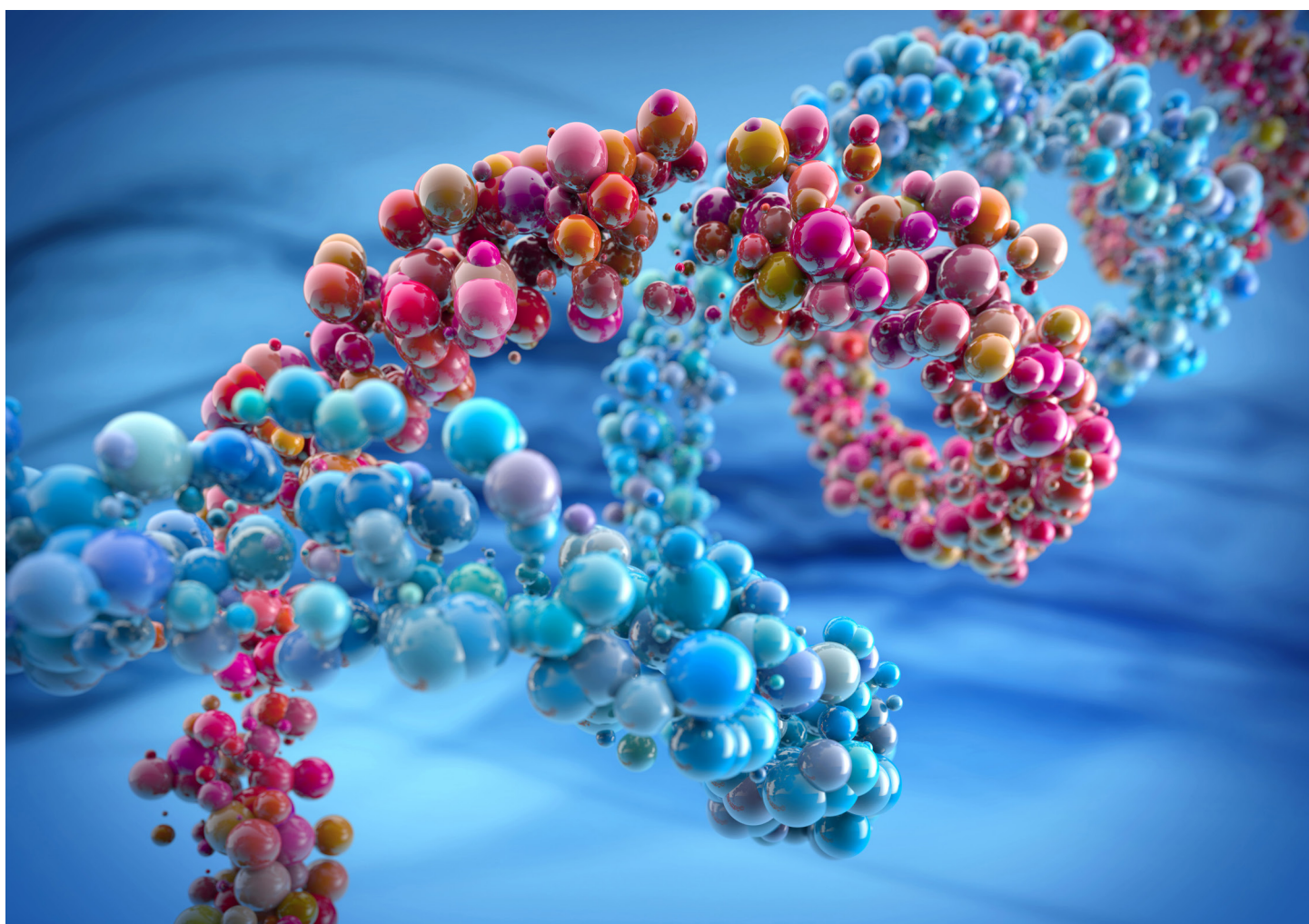




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RESEARCH PRODUCTS

# Stable Isotopes for Biomolecular NMR



**Cambridge Isotope Laboratories, Inc.**

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## Welcome

Over the years, Cambridge Isotope Laboratories, Inc (CIL) has grown to be the world's leader in production and distribution of stable isotope-labeled compounds for biological NMR applications. CIL has partnered with the scientific community in the last decade to enable significant developments in isotopic labeling of proteins and nucleic acids for magnetic resonance applications by commercializing novel compounds and reagents.

CIL is pleased to present this catalog for biomolecular NMR. Here you will find the latest in isotope-labeled products to support your research, along with contributions written by some of the world's most preeminent NMR spectroscopists. We are particularly pleased to present pieces written by Gerhard Wagner, Lewis Kay, Masatsune Kainosho, Leonid Brown and Vladimir Ladizhansky, Hanudatta Atreya, Tim Cross, and Robert Griffin.

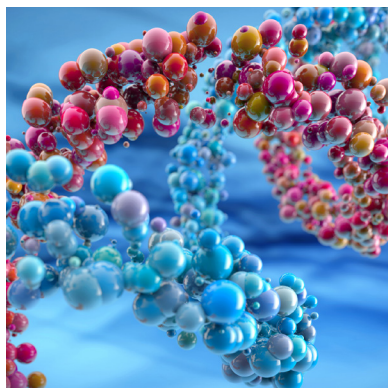
Also included in this catalog is a summation of CIL application notes relevant to magnetic resonance studies of biological molecules and systems. These application notes help illustrate the usefulness of our products for both solution and solid-state NMR applications. We would like to thank the many researchers who have written application notes. Also included is our most recent application note written by Rui Huang and Lewis Kay titled "Stereospecific Leu/Val Methyl Labeling: An Important Technology for NMR Studies of High-Molecular-Weight Complexes" (Application Note 48).

As new technologies and applications to study biopolymers using NMR advance, CIL continues to maintain a leadership role in developing new products for the benefit of the entire biomolecular NMR community. CIL welcomes suggestions from our customers on additional products that would be beneficial to their research. It has been through our partnerships and close relationships with our customers over the past 35 years that we have been able to significantly expand our product offering in order to assist the biomolecular NMR community in the advancement of their studies utilizing stable isotope-labeled compounds as a tool for NMR discovery.

All of us at CIL appreciate the collaborative relationships we have with our customers. We look forward to continuing to work with you to expand our product offerings in ways that will best fulfill your needs.

Kevin Millis, PhD  
Senior Scientist  
Applications Development

*You can find all of the information presented in this catalog on [isotope.com](http://isotope.com). Visitors to our website can immediately access updated product information and documentation, such as a certificate of analysis (CoA) and safety data sheets (SDS) and can also find real-time product availability. Visit [isotope.com](http://isotope.com) to learn more.*



Cambridge Isotope Laboratories (CIL) is the world leader in the separation and manufacture of stable isotopes and stable isotope-labeled compounds. For over 35 years, CIL has remained the premier supplier of stable isotopes for NMR and MRS/MRI research applications. These products include a diverse line of RNA/DNA products, minimal media reagents (carbohydrates, ammonium salts), cell-free expression reagents and kits, free and protected amino acids, as well as cell-growth media for eukaryotic and prokaryotic cell lines. Additionally, CIL offers a comprehensive line of deuterated solvents, detergents, and buffers. Our products have been specifically designed and tested with the most discerning NMR spectroscopist and structural biologist in mind. CIL actively supports the world's NMR community through meeting sponsorships and customer collaborations.

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- Canadian shipments will be sent via FedEx or truck.
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## Utility of Stable Isotopes in Structural Biology

**Investigator  
Spotlight****Gerhard Wagner, PhD**

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts USA

The use of stable isotopes has empowered NMR to become a major player in mechanistic structural biology and drug discovery. Over the past quarter century introduction of new isotope-labeling procedures has led to several quantum leaps in the advancement of NMR spectroscopy. By introducing new NMR active nuclei, or replacing protons with deuterons, isotope labeling is used to obtain new correlations and to reduce complexity of spectra. Thus, it has tremendously facilitated obtaining specific structural and dynamic information. New isotope-labeling procedures have stimulated the development of a plethora of new NMR experiments. Today, labeling is an integral element of current state-of-the-art NMR technology and can be expected to play a major role in future developments. While new labeling methods typically originate from academic laboratories, it is usually not economical to cultivate procedures for every isotope-producing procedure in all interested laboratories with student and post-doc members. This is best pursued by companies, such as CIL, that can provide sustainable long-term isotope commodities to the scientific community and are an invaluable service for the NMR community.

### Today, labeling is an integral element of current state-of-the-art NMR technology and can be expected to play a major role in future developments.

Until the early 1970s NMR spectroscopy was a method primarily used by chemists for characterizing small molecules, checking chemical structures, and purity of reaction products. Studies of proteins and nucleic acids became feasible with the arrival of high-resolution superconducting magnets at higher field and new consoles for pulsed NMR, development of double-resonance experiments, intelligent use of the nuclear Overhauser effect, and eventually the introduction of 2D NMR. This allowed collection of numerous structural parameters, and with the development of new computer programs, the first solution structures were determined with NMR in the early to mid 1980s. However, the method was limited to proteins up to 10 kDa or 15 kDa, and the process was very time consuming.

Fortunately, techniques were developed for producing isotope-labeled proteins in bacterial expression systems.<sup>1,2</sup> The availability of <sup>15</sup>N labeled proteins enabled a first quantum leap. Three-dimensional <sup>15</sup>N-dispersed NOESY experiments were developed leading to a tremendous gain in signal dispersion and spectral simplification. Larger structures could be obtained in shorter time and with higher precision. Access to routine production of <sup>15</sup>N and <sup>13</sup>C-labeled proteins has also opened an entirely new field of protein dynamics, which is thriving ever since. It has provided and is yielding new insights into how proteins fluctuate and perform functions, such as in enzymes.

The possibility to produce <sup>15</sup>N/<sup>13</sup>C labeled proteins has made possible the development of triple-resonance experiments for conformation-independent sequential assignments. These experiments caused a second quantum leap in protein NMR and made assignments more reliable and very efficient. Triple resonance experiments form today the basis of all assignments of proteins.

Another quantum leap was the introduction of deuteration by over-expressing proteins in <sup>2</sup>H<sub>2</sub>O, which reduces dipolar broadening of resonances by proton dilution.<sup>3</sup> Spectroscopy is essentially done with the amide protons that can be reintroduced by dissolving deuterated proteins in H<sub>2</sub>O for measurements. The reduction in line-widths of amide proteins was dramatic and excited everyone in the field when this was introduced. Deuteration is a key requirement for the performance of TROSY (Transverse Relaxation Optimized Spectroscopy) experiments, which brought another performance leap and is today used for nearly all NMR studies of larger proteins.

With deuterated proteins, backbone assignments are rather routine even for rather large proteins. The challenge is to have a sufficiently large number of probes for measuring distance constraints. Thus, introduction of selectively protonated groups into deuterated proteins has become very important. Most prominent is the introduction of protonated ILV methyls, which uses <sup>13</sup>C  $\alpha$ -ketoisovalerate and  $\alpha$ -ketobutyrate as precursors for labeling. There is a wide range of applications to introduce protonated groups into deuterated proteins beyond ILV labeling. Most sophisticated is the SAIL (stereo-arrayed isotope labeling) approach, which minimizes dipolar broadening while maintaining a large number of protons for distance measurements.

In our laboratory, the use of ILV labeling in a deuterated background, spiked with few protonated residues, has allowed solving several large structures, where the prime source of distance constraints came from methyl-methyl and methyl-aromatic contacts. The clear separation of the methyl signals from the amides as the only protons made possible recording multiple high-resolution 4D NOESYs in a time-shared manner within a reasonable time.<sup>4</sup> Figure 1 shows the structure of the enterobactin synthase 37 kDa EntF T-TE di-domain where most distance restraints were obtained from such isotope-labeled samples.<sup>5</sup>

Recent developments have explored additional possibilities for the use of isotopes to enhance NMR spectroscopy. This includes the advent of new cryogenic probes that are optimized for <sup>13</sup>C and <sup>15</sup>N direct detection. First used for direct detection of carbonyl carbons, direct detection seemed primarily suited for paramagnetic proteins where carbons could be observed closer to the paramagnetic center

*Continued* ➤

## Utility of Stable Isotopes in Structural Biology (continued)

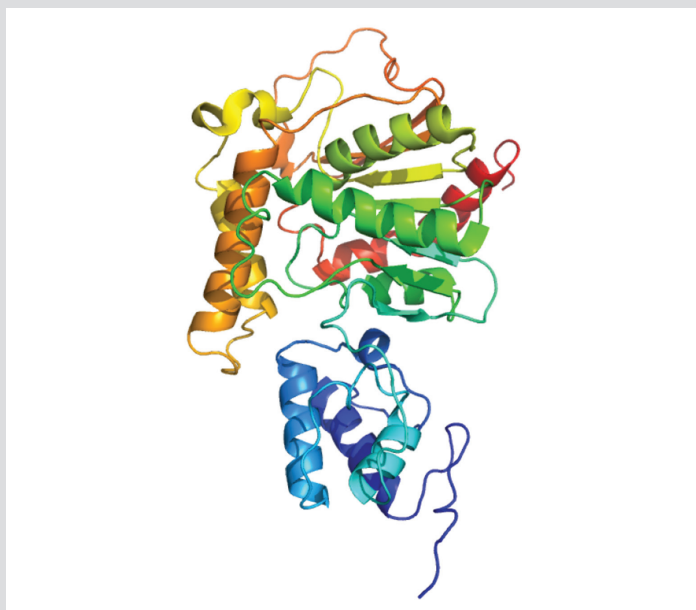


Figure 1. Structure of the 37 kDa EntF T-TE di-domain.<sup>5</sup>

than protons. However, it became obvious that direct  $^{13}\text{C}$  detection can be quite powerful for any protein when using alternate  $^{13}\text{C}$ - $^{12}\text{C}$  labeling, which can be obtained when using  $[2\text{-}^{13}\text{C}]$  or  $[1,3\text{-}^{13}\text{C}_2]$  glycerol as a carbon source as originally described by LeMaster.<sup>6</sup> This eliminates most one-bond carbon-carbon couplings. When the protein is deuterated in addition, carbon resonances become extremely sharp and new long-range correlations can be observed through backbone and side chain nuclei.<sup>7,8</sup> This is particularly interesting for assigning prolines, which are often found adjacent to phosphorylation sites in regulatory domains of proteins and are difficult to characterize with traditional NMR methods. It is likely that future developments of isotope labeling will enhance this approach.

Currently, the biggest hurdle in isotope-labeling proteins for NMR studies is the lack of efficient methods for producing isotope-labeled proteins in eukaryotic or mammalian expression systems. Many mammalian proteins of interest are misfolded when expressed in bacterial systems due to the lack of folding machinery that is present in eukaryotic cells, such as those for forming disulfides. Production in insect cells, yeast, or CHO (Chinese hamster ovary) cells is possible using special growth media, but the process is still far from being efficient for wide use.

Compared to proteins, the use of isotopes has been slower in NMR of nucleic acids. Due to a lower proton density, dipolar broadening is less of a problem in nucleic acids. Also connectivity through phosphorus is inefficient because of the large chemical shift anisotropy. Thus, assignments are mainly made with NOE correlations. However, novel labeling methods<sup>9</sup> and intelligent use of inserting deuterated nucleotides has made tremendous impact for structural studies of large nucleic acids.<sup>10</sup>

Considering the great impact isotope labeling had in the development of NMR in the past, it can be anticipated that it will play an equal or even greater role in the future.

### Acknowledgement

Large amounts of stable isotopes were purchased with support from the NIH grant 047467.

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## Minimal Media Reagents

The *E. coli* expression system using minimal media is the most common means of overexpressing isotope-enriched recombinant protein for NMR investigations, primarily because the use of minimal media is the most economical way to grow isotope-labeled bacteria. Minimal media is a mixture M9 salts, glucose (as the sole carbon source), and ammonium chloride or ammonium sulfate (as the sole nitrogen source) that is formulated in either H<sub>2</sub>O or D<sub>2</sub>O. Often, depending on the *E. coli* strain used, additional reagents, such as cofactors, are added to the media to enhance growth.

For proteins greater than ~25 kDa in size, deuteration is generally required to simplify spectra and reduce the deleterious effects of line-broadening associated with <sup>1</sup>H dipolar coupling. Therefore, minimal media used to express such proteins must be formulated using D<sub>2</sub>O. For the investigations of large proteins or protein complexes, fully deuterated glucose as the carbon source is required. In particular, it is advantageous to use deuterated glucose in conjunction with the ILV selective methyl-labeling strategies that require a highly deuterated protein. The use of protonated <sup>13</sup>C<sub>6</sub> glucose as the carbon source may still be acceptable in some situations, because the level of incorporation of deuterium from the solvent into the expressed proteins is still significant (e.g., ~70-80%). <sup>13</sup>C<sub>6</sub> glucose in minimal media formulated in D<sub>2</sub>O may also be used to label all protein methyl groups,<sup>1</sup> although CHD<sub>2</sub>-detected variants must be used to remove signals from the CH<sub>3</sub> and CH<sub>2</sub>D isotopomers in <sup>1</sup>H-<sup>13</sup>C correlation spectra.

To aid in handling multiple simultaneous growths, auto-induction methods have been developed which utilize minimal media containing glucose, glycerol, and lactose. Auto-induction methods utilize the preference of *E. coli* containing *lac* operon-derived expression systems (e.g., pET, pGEM, and pQE) to selectively use different carbon sources during diauxic growth. Hence, auto-induction methods will lead to growth on alternative carbon sources such as glycerol or lactose after exhaustion of glucose in the growth medium. The basis for auto-induction and adaptation to <sup>13</sup>C,<sup>15</sup>N was reported by Studier<sup>2</sup> and further optimized for high-throughput production by Tyler, et al.<sup>3</sup>

Although minimal media is the most economical to label *E. coli*, the resulting growth rate and expression levels are sometimes not suitable to conduct the desired studies. An alternate method used to enhance protein yield and growth characteristics is to spike low levels of suitably labeled rich media into minimal media prior to induction. Please see CIL Application Note 12 at isotope.com for additional information.

The table below shows which labeled reagents to use in minimal media to obtain the highest level of incorporation of the desired labeling pattern.

**Table:** Minimal media reagents used to express single-, double- and triple-labeled protein.

Desired Labeling Pattern	<sup>15</sup> N NH <sub>4</sub> Cl or ( <sup>15</sup> NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	D-Glucose ( <sup>13</sup> C <sub>6</sub> )	D-Glucose ( <sup>13</sup> C <sub>6</sub> ; D <sub>7</sub> )	D-Glucose (D <sub>7</sub> )	D <sub>2</sub> O
<sup>15</sup> N	+	-	-	-	-
<sup>13</sup> C	-	+	-	-	-
D	-	-	-	+	+
<sup>13</sup> C, <sup>15</sup> N	+	+	-	-	-
<sup>15</sup> N, D	+	-	-	+	+
<sup>13</sup> C, D	-	-	+	-	+
<sup>13</sup> C, <sup>15</sup> N, D	+	-	+	-	+

### Minimal Media Reagents for Uniform Labeling

Catalog No.	Description
CLM-1396	D-Glucose ( <sup>13</sup> C <sub>6</sub> , 99%)
DLM-2062	D-Glucose (1,2,3,4,5,6,6-D <sub>7</sub> , 97-98%)
CDLM-3813	D-Glucose ( <sup>13</sup> C <sub>6</sub> , 99%; 1,2,3,4,5,6,6-D <sub>7</sub> , 97-98%)
CLM-1510	Glycerol ( <sup>13</sup> C <sub>3</sub> , 99%)
DLM-558	Glycerol (D <sub>8</sub> , 99%)
NLM-467	Ammonium chloride ( <sup>15</sup> N, 99%)
NLM-713	Ammonium sulfate ( <sup>15</sup> N <sub>2</sub> , 99%)
DLM-4-99	Deuterium oxide (D, 99%)
DLM-4-99.8	Deuterium oxide (D, 99.8%)
DLM-4	Deuterium oxide (D, 99.9%)

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## Methyl Labeling

Protonated  $^{13}\text{C}$  methyl groups in deuterated proteins are excellent probes for structure and dynamics, and are especially crucial to study high-molecular weight proteins and protein complexes. The popular ILV labeling strategy utilizes labeled biosynthetic precursors to produce protein containing either uniformly or selectively labeled isoleucine, leucine, and valine.  $\alpha$ -Ketoisobutyrate is the precursor to Ile and may be used to label the  $\delta^1$  methyl in Ile.  $\alpha$ -Ketoisovalerate is the precursor to Leu and Val and is used to label one or both methyl groups in these amino acids. Please see CIL application notes 16 and 25 at [isotope.com](http://isotope.com) for more information regarding this application.

CIL is pleased to offer a large selection of  $\alpha$ -ketobutyric and  $\alpha$ -ketoisovaleric acids, along with pyruvate, for use in selective

methyl and side-chain labeling. Uniform  $^{13}\text{C}$ -labeled forms of the precursors are used in conjunction with  $^{13}\text{C}_6$  glucose to produce uniform  $^{13}\text{C}$ -labeled Ile and Leu.

CIL also offers kits of conveniently packaged reagents for labeling proteins with isoleucine, leucine, valine, and alanine (i.e., "ILVA" labeling) or alanine only. These kits are for use with 1 L amounts of deuterated minimal media.<sup>1,2</sup> Please see CIL Application Note 25 at [isotope.com](http://isotope.com) for more details regarding ILVA and alanine labeling. Methionine (2,3,3,4,4- $\text{D}_5$ , methyl- $^{13}\text{CH}_3$ ) and threonine (4- $^{13}\text{C}$ ; 2,3- $\text{D}_2$ ) are also available for use with deuterated minimal media to provide new methyl probes in addition to Leu, Ile, Val, and Ala.

### $\alpha$ -Keto Acids

Catalog No.	Description	Unit Size
CLM-6820	$\alpha$ -Ketobutyric acid, sodium salt (methyl- $^{13}\text{C}$ , 99%)	0.5 g
CLM-6164	$\alpha$ -Ketobutyric acid, sodium salt ( $^{13}\text{C}_4$ , 98%)	0.5 g
DLM-9168	$\alpha$ -Ketobutyric acid, sodium salt (3,3- $\text{D}_2$ , 98%)	Please inquire
CDLM-4611	$\alpha$ -Ketobutyric acid, sodium salt ( $^{13}\text{C}_4$ , 98%; 3,3- $\text{D}_2$ , 98%)	0.1 g, 0.25 g
CDLM-7318	$\alpha$ -Ketobutyric acid, sodium salt (methyl- $^{13}\text{C}$ , 99%; 3,3- $\text{D}_2$ , 98%)	0.5 g, 1 g
CDLM-7353	$\alpha$ -Ketobutyric acid, sodium salt (4- $^{13}\text{C}$ , 99%; 3,3,4,4- $\text{D}_4$ , 98%)	0.25 g
CLM-6821	$\alpha$ -Ketoisovaleric acid, sodium salt (dimethyl- $^{13}\text{C}_2$ , 99%)	0.5 g
CLM-4418	$\alpha$ -Ketoisovaleric acid, sodium salt ( $^{13}\text{C}_5$ , 98%)	0.25 g, 1 g
DLM-4646	$\alpha$ -Ketoisovaleric acid, sodium salt ( $\text{D}_7$ , 98%)	Please inquire
CDLM-4418	$\alpha$ -Ketoisovaleric acid, sodium salt ( $^{13}\text{C}_5$ , 98%; 3- $\text{D}$ , 98%)	0.25 g
CDLM-7317	$\alpha$ -Ketoisovaleric acid, sodium salt (3-methyl- $^{13}\text{C}$ , 99%; 3,4,4,4- $\text{D}_4$ , 98%)	0.5 g, 1 g
CDLM-7354	$\alpha$ -Ketoisovaleric acid, sodium salt (3-methyl- $^{13}\text{C}$ , 99%; 3-methyl- $\text{D}_2$ , 3,4,4,4- $\text{D}_4$ , 98%)	0.25 g
CDLM-10647	$\alpha$ -Ketoisovaleric acid, sodium salt (dimethyl- $^{13}\text{C}_2$ , 99%; 3- $\text{D}$ , 98%)	0.1 g, 0.5 g
CDLM-8446	$\alpha$ -Ketoisovaleric acid, sodium salt (dimethyl- $^{13}\text{C}_2$ , 98%; 3-methyl- $\text{D}_2$ , 4,4- $\text{D}_2$ , 98%)	0.25 g
CDLM-8100	$\alpha$ -Ketoisovaleric acid, sodium salt (1,2,3,4- $^{13}\text{C}_4$ , 99%; 3,4',4',4'- $\text{D}_4$ , 97-98%)	0.25 g
CLM-1575	Sodium pyruvate (3- $^{13}\text{C}$ , 99%)	0.1 g, 0.5 g, 1 g
CLM-2440	Sodium pyruvate ( $^{13}\text{C}_3$ , 99%)	0.5 g, 1 g

### Amino Acids

Catalog No.	Description	Unit Size
CDLM-8649	L-Alanine (3- $^{13}\text{C}$ , 99%; 2- $\text{D}$ , 96%)	1 g
CLM-206	L-Methionine (methyl- $^{13}\text{C}$ , 99%)	1 g
CDLM-8885	L-Methionine (2,3,3,4,4- $\text{D}_5$ , 98%; methyl- $^{13}\text{CH}_3$ , 99%)	0.5 g, 1 g
CDLM-9307	L-Threonine (4- $^{13}\text{C}$ , 97%; 2,3- $\text{D}_2$ , 96-98%)	0.1 g, 0.5 g
CLM-9217	L-Valine (dimethyl- $^{13}\text{C}_2$ , 99%)	0.25 g, 1 g

### CDLM-8805-KIT – *in vivo* Alanine Methyl-Labeling Kit

Catalog No.	Kit Components (four vials)	Unit Size
DLM-584	Succinic acid ( $\text{D}_4$ , 98%)	2.5 g
DLM-4646	$\alpha$ -Ketoisovaleric acid, sodium salt ( $\text{D}_7$ , 98%)	0.2 g
DLM-141	L-Isoleucine ( $\text{D}_{10}$ , 98%)	0.06 g
CDLM-8649	L-Alanine (3- $^{13}\text{C}$ , 99%; 2- $\text{D}$ , 96%)	0.8 g

The materials in this kit are to be used in conjunction with 1 L of deuterated minimal media.<sup>1,2</sup> 1 L of minimal media that is suitable for use with this product typically contains 2 g of D-glucose- $\text{D}_7$ , 1 g of ammonium salt, and 11.3 g of M9 salts in  $\text{D}_2\text{O}$ .

CDLM-8806-KIT – *in vivo* ILVA Methyl-Labeling Kit

Catalog No.	Kit Components (four vials)	Unit Size
DLM-584	Succinic acid (D <sub>4</sub> , 98%)	2.5 g
CDLM-7317	$\alpha$ -Ketoisovaleric acid, sodium salt (methyl- <sup>13</sup> C, 99%; 3,4,4,4-D <sub>4</sub> , 98%)	0.12 g
CDLM-7318	$\alpha$ -Ketobutyric acid, sodium salt (methyl- <sup>13</sup> C, 99%; 3,3-D <sub>2</sub> , 98%)	0.06 g
CDLM-8649	L-Alanine (3- <sup>13</sup> C, 99%; 2-D, 96%)	0.8 g

The materials in this kit are to be used in conjunction with 1 L of deuterated minimal media.<sup>1</sup> 1 L of minimal media that is suitable for use with this product typically contains 2 g of D-glucose-D<sub>7</sub>, 1 g of ammonium salt, and 11.3 g of M9 salts in D<sub>2</sub>O.

## References

1. Ayala, I.; Sounier, R.; Use, N.; et al. **2009**. An efficient protocol for the complete incorporation of methyl-protonated alanine in perdeuterated protein. *J Biomol NMR*, 43(2), 111-119.
2. Isaacson, R.L.; Simpson, P.J.; Liu, M.; et al. **2007**. A new labeling method for methyl transverse relaxation-optimized spectroscopy NMR spectra of alanine residues. *J Am Chem Soc*, 129(50), 15428-15429.

## Alanine Probes of Supramolecular Structure and Dynamics

Investigator  
Spotlight

Lewis Kay, PhD

Department of Chemistry, University of Toronto, Ontario, Canada

The development of new protein labeling strategies, along with optimized experiments that exploit the label, have significantly impacted on the types of biochemical problems that can now be addressed by solution NMR spectroscopy. One popular strategy in studies of high molecular weight proteins involves the use of a pair of  $\alpha$ -keto acids,  $\alpha$ -ketobutyrate and  $\alpha$ -ketoisovalerate, which serve as the biosynthetic precursors for the production of Ile and Leu/Val, respectively.<sup>1</sup> Addition of these precursors to highly deuterated protein expression media produces <sup>2</sup>H, Ile, Leu, Val- methyl-labeled proteins. These precursors are available with different methyl isotopomers (<sup>13</sup>CH<sub>3</sub>, <sup>13</sup>CH<sub>2</sub>D, <sup>13</sup>CHD<sub>2</sub>) so that a large variety of labeled proteins can be produced<sup>2</sup> and a correspondingly large number of experiments can be performed using <sup>1</sup>H, <sup>13</sup>C or <sup>2</sup>H nuclei.

**The development of new protein labeling strategies, along with optimized experiments that exploit the label, have significantly impacted on the types of biochemical problems that can now be addressed by solution NMR spectroscopy.**

Ile, Leu, Val methyl groups are powerful probes of side-chain structure and dynamics, and their utility has been described in a significant number of papers.<sup>2</sup> Ala methyls, on the other hand, report on properties of the backbone and hence provide important complementary information. A number of recent publications outline approaches for the production of highly deuterated, Ala-[<sup>13</sup>CH<sub>3</sub>] labeled proteins.<sup>3,4</sup> Ala labeling is challenging since this residue is produced directly as a result of transamination of pyruvate, which is also a precursor in the production of the branched-chain amino acids. Transamination is reversible so even if free methyl-labeled Ala is provided to the media, scrambling will

occur with label incorporated at a variety of potentially undesired locations. Recently Boisbouvier and coworkers have developed a procedure to generate methyl labeling at Ala side chains with minimal (<1%) scrambling.<sup>3</sup> This was achieved by adding 2-[<sup>2</sup>H],3-[<sup>13</sup>C]-Ala (800 mg/L) as well as precursors for other pathways in which the scrambled amino acids are produced.

The ability to produce highly deuterated, Ala-[<sup>13</sup>CH<sub>3</sub>] labeled proteins further increases the number of methyl probes available for studies of very high molecular weight systems.<sup>5</sup> A number of applications involving Ala methyl probes can be envisioned, including measurement of backbone dynamics through relaxation studies, probing structure via residual dipolar couplings, methyl-methyl NOEs (nuclear Overhauser effect) or PREs (paramagnetic relaxation enhancement) and studies of molecular interactions.

## References

1. Goto, N.K.; Gardner, K.H.; Mueller, G.A.; et al. **1999**. A robust and cost-effective method for the production of Val, Leu, Ile ( $\delta$ 1) methyl-protonated <sup>15</sup>N-, <sup>13</sup>C-, <sup>2</sup>H-labeled proteins. *J Biomol NMR*, 13(4), 369-374.
2. Tugarinov, V.; Kay, L.E. **2005**. Methyl groups as probes of structure and dynamics in NMR studies of high-molecular-weight proteins. *ChemBioChem*, 6(9), 1567-1577.
3. Ayala, I.; Sounier, R.; Use, N.; et al. **2009**. An efficient protocol for the complete incorporation of methyl-protonated alanine in perdeuterated protein. *J Biomol NMR*, 43(2), 111-119.
4. Isaacson, R.L.; Simpson, P.J.; Liu, M.; et al. **2007**. A new labeling method for methyl transverse relaxation-optimized spectroscopy NMR spectra of alanine residues. *J Am Chem Soc*, 129(50), 15428-15429.
5. Amero, C.; Schanda, P.; Dura, M.A.; et al. **2009**. Fast two-dimensional NMR spectroscopy of high molecular weight protein assemblies. *J Am Chem Soc*, 131(10), 3448-3449.

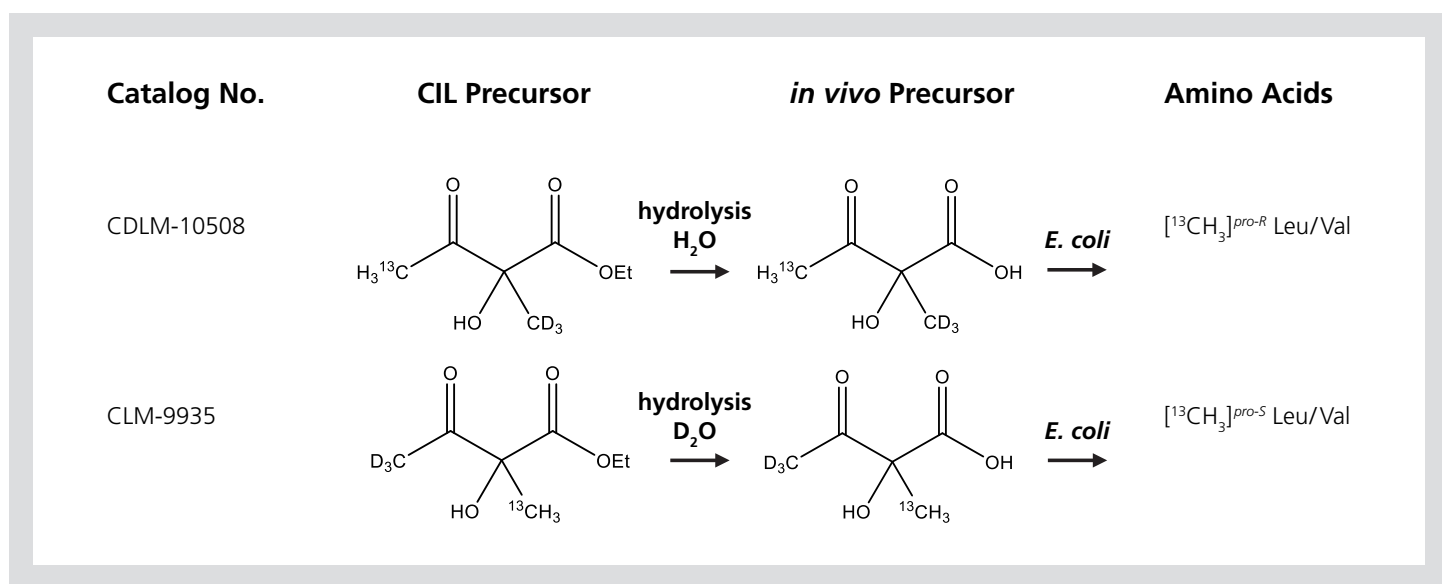
## Prochiral Precursors

The use of methyl-labeled acetolactate as an *in vivo* prochiral precursor for the biosynthesis of *proR*- or *proS*- $^{13}\text{CH}_3$  valine and leucine in *E. coli* cells has been developed by Jerome Boissbouvier and colleagues.<sup>1</sup> Using acetolactate precursors instead of  $\alpha$ -ketoisovaleric acid as a metabolic precursor reduces the number of labeled methyl cross-peaks by a factor of two. The resulting simplification of the methyl region of the acquired spectra makes the study of larger proteins possible and/or more manageable. In addition, the signal-to-noise ratios for the methyl cross peaks when using a prochiral precursor are twice as great when compared to using  $\alpha$ -ketoisovaleric acid.

CIL offers an alternative to acetolactate as a prochiral precursor. These precursors come as ethyl esters and require the user to perform a hydrolysis step prior to use. Instructions on how to perform the hydrolysis step are included with each order.

Catalog No.	Description	Package Size	Media Volume
CDLM-10508-0.25	Ethyl 2-hydroxy-2-methyl-3-oxobutanoate (4- $^{13}\text{C}$ , 99%; 2-methyl- $\text{D}_3$ , 98%) ( <i>proR</i> )	0.25 g	1 L
CDLM-10508-1	Ethyl 2-hydroxy-2-methyl-3-oxobutanoate (4- $^{13}\text{C}$ , 99%; 2-methyl- $\text{D}_3$ , 98%) ( <i>proR</i> )	1 g	4 L
CLM-9935-0.25	Ethyl 2-hydroxy-2-methyl-3-oxobutanoate (methyl- $^{13}\text{C}$ , 99%) ( <i>proS</i> )	0.25 g	1 L
CLM-9935-1	Ethyl 2-hydroxy-2-methyl-3-oxobutanoate (methyl- $^{13}\text{C}$ , 99%) ( <i>proS</i> )	1 g	4 L

### Production of Pro-R and Pro-S $^{13}\text{CH}_3$ Labeled Leucine and Valine Using CIL Precursors



#### Reference

- Gans, P.; Hamelin, O.; Sounier, R.; et al. **2010**. Stereospecific isotopic labeling of methyl groups for NMR spectroscopic studies of high molecular weight proteins. *Angew Chem Int Ed Engl*, 49(11), 1958-1962.

## Rich *E. coli* Media

Although growth in minimal (M9) media is economical, there is no substitute for the enhanced growth rates and increased levels of protein expression that may be gained by the use of a rich medium. Rich bacterial media are complex formulations that are usually

derived from algal hydrolysates and contain all the necessary nutrients to promote excellent growth. CIL offers a number of rich media used in labeled protein expression using bacterial systems.

### BioExpress® 1000

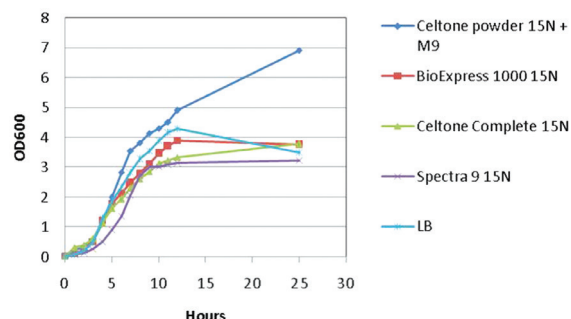
BioExpress 1000 is CIL's all-time classic rich bacterial cell growth medium. BioExpress 1000 provides excellent growth and expression characteristics for a number of different bacterial systems. BioExpress 1000 contains nearly the same level of amino acids as LB medium. Glucose levels range from 0.1-0.5 g/L, depending on the batch. BioExpress 1000 media is prepared by adding sterile cell culture-grade water and mixing. Please note that D<sub>2</sub>O is required for reconstitution for products containing deuterium. BioExpress 1000 is supplied as a 100 mL sterile liquid 10× concentrate, and reconstitutes to 1 L with no final pH adjustment required; 10 mL sample sizes are also available. The 10 mL sample size reconstitutes to make 100 mL of media with no final pH adjustment required. A small amount of solid material may be present after dilution of the BioExpress 1000 with water. This material should be removed by using the supplied 0.2 µm filter prior to use.

Catalog No.	Description
CGM-1000-C	BioExpress 1000 ( <sup>13</sup> C, 98%) 10× concentrate
CGM-1000-D	BioExpress 1000 (D, 98%) 10× concentrate
CGM-1000-N	BioExpress 1000 ( <sup>15</sup> N, 98%) 10× concentrate
CGM-1000-CD	BioExpress 1000 ( <sup>13</sup> C, 98%; D, 98%) 10× concentrate
CGM-1000-CN	BioExpress 1000 ( <sup>13</sup> C, 98%; <sup>15</sup> N, 98%) 10× concentrate
CGM-1000-DN	BioExpress 1000 (D, 98%; <sup>15</sup> N, 98%) 10× concentrate
CGM-1000-CDN	BioExpress 1000 ( <sup>13</sup> C, 98%; <sup>15</sup> N, 98%; D, 98%) 10× concentrate
CGM-1000-U	BioExpress 1000 (unlabeled) 10× concentrate

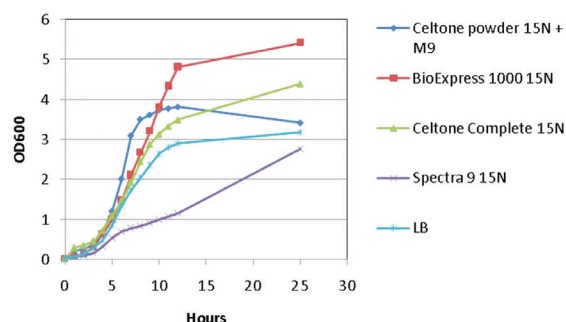
For pointers on how to maximize protein yield using CIL's BioExpress 1000 media, please see CIL Application Note 15 at [isotope.com](http://isotope.com). To learn how spiking BioExpress 1000 media into minimal media provides a low-cost means to enhance the performance of minimal media, please see CIL Application Note 12 at [isotope.com](http://isotope.com).

BioExpress® is a registered trademark of Cambridge Isotope Laboratories, Inc.

Growth Curves *E. coli* (BL21, pGFP)



Growth Curves *E. coli* (M15, pTMK)



“In our hands, CIL's BioExpress 1000 worked like a charm.

The cell growth rate and protein expression level essentially matched the results obtained with Luria broth, and the <sup>15</sup>N labeling efficiency was excellent.”

Tero Pihlajamaa, PhD  
Institute of Biotechnology  
University of Helsinki, Finland



## Rich *E. coli* Media

### Celtone® and Celtone® Plus Powder

Celtone powder is CIL's most flexible nutrient-rich media. The advantage of Celtone powder is that researchers can formulate a custom medium based on their specific research needs. Depending on cell line and desired performance, this powdered media can be used at concentrations ranging from 1 g to 10 g per liter. Truly exceptional performance has been achieved using 10 g of Celtone powder in 1 L of medium containing M9 salts, 2-3 g glucose and 1 g of ammonium chloride (see graph on page 13). Because it is a powder, this product has the longest shelf life of any fully rich bacterial cell growth medium.

Celtone powder is available in 0.5 g and 1 g packaged sizes.

Celtone Plus base powder contains uniformly enriched amino acids and peptides along with a collection of nutrients that promote robust growth for BL21 cells. Celtone Plus contains over twice the nutrients of Celtone, providing the highest levels of protein expression yields of any CIL rich bacterial cell growth media. Celtone Plus is added to minimal media at concentrations from 1 to 10 g/L.

Celtone Plus is available in 1 g and 10 g packaged sizes.

Catalog No.	Description
CGM-1030P-C	Celtone Base Powder ( <sup>13</sup> C, 98%)
CGM-1030P-D	Celtone Base Powder (D, 97%)
CGM-1030P-N	Celtone Base Powder ( <sup>15</sup> N, 98%)
CGM-1030P-CN	Celtone Base Powder ( <sup>13</sup> C, 98%; <sup>15</sup> N, 98%)
CGM-1030P-DN	Celtone Base Powder (D, 97%; <sup>15</sup> N, 98%)
CGM-1030P-CDN	Celtone Base Powder ( <sup>13</sup> C, 98%; D, 97%; <sup>15</sup> N, 98%)
CGM-1030P-U	Celtone Base Powder (unlabeled)
CGM-1050P-C	Celtone Plus Base Powder ( <sup>13</sup> C, 97-99%)
CGM-1050P-D	Celtone Plus Base Powder (D, 97%+)
CGM-1050P-N	Celtone Plus Base Powder ( <sup>15</sup> N, 97-99%)
CGM-1050P-DN	Celtone Plus Base Powder (D, 97-99%; <sup>15</sup> N, 97-99%)
CGM-1050P-CDN	Celtone Plus Base Powder ( <sup>13</sup> C, 97-99%; D, 97-99%; <sup>15</sup> N, 97-99%)
CGM-1050P-U	Celtone Plus Base Powder (unlabeled)

**Note:** If deuterium labeling is desired, D<sub>2</sub>O must be used in media preparation.

It is normal to have insoluble material present after dissolution. This material may be removed using filter paper prior to sterile filtration and will not affect performance of the medium.

### Celtone® Complete

Celtone Complete yields a growth rate comparable to LB media, allowing for inoculation and induction within one working day. Glucose levels range from ~0.3 to 0.6 g/L, depending on the batch. Celtone Complete contains 10 g of Celtone powder per liter of media; it is ready-to-use and does not require dilution or pH adjustment. Each lot is tested for sterility, cell growth and protein expression. Celtone Complete is available in 0.1 L and 1 L sizes.

Catalog No.	Description
CGM-1040-C	Celtone Complete Medium ( <sup>13</sup> C, 98%)
CGM-1040-D	Celtone Complete Medium (D, 97%)
CGM-1040-N	Celtone Complete Medium ( <sup>15</sup> N, 98%)
CGM-1040-CN	Celtone Complete Medium ( <sup>13</sup> C, 98%; <sup>15</sup> N, 98%)
CGM-1040-DN	Celtone Complete Medium (D, 97%; <sup>15</sup> N, 98%)
CGM-1040-CDN	Celtone Complete Medium ( <sup>13</sup> C, 98%; D, 97%; <sup>15</sup> N, 98%)
CGM-1040-U	Celtone Complete Medium (unlabeled)

### Spectra 9 Media

Spectra 9 Media is not a fully rich medium, however, it represents a cost-effective medium for *E. coli* growth and protein expression. This ready-to-use liquid medium is comprised of labeled salts, labeled carbohydrates (>2 g/L glucose), and contains Celtone powder at a concentration of 1 g/L.

Catalog No.	Description
CGM-3030-C	Spectra 9 ( <sup>13</sup> C, 98%)
CGM-3030-D	Spectra 9 (D, 97%)
CGM-3030-N	Spectra 9 ( <sup>15</sup> N, 98%)
CGM-3030-CN	Spectra 9 ( <sup>13</sup> C, 98%; <sup>15</sup> N, 98%)
CGM-3030-DN	Spectra 9 (D, 97%; <sup>15</sup> N, 98%)
CGM-3030-CDN	Spectra 9 ( <sup>13</sup> C, 98%; D, 97%; <sup>15</sup> N, 98%)
CGM-3030-U	Spectra 9 (unlabeled)

BioExpress® is a registered trademark of Cambridge Isotope Laboratories, Inc.  
Celtone® is a registered trademark of Cambridge Isotope Laboratories, Inc.

“The customer service at Cambridge Isotope Labs is unmatched. Our dedicated sales representative responds promptly and thoroughly with quotes, product information, shipping information, services, and other information. Their products are of the highest quality, and we continue to trust their chemicals and service. Any issue has been resolved quickly and painlessly. For these reasons we have been dedicated to CIL.”

Victor Beaumont, PhD student  
Loria Group  
Department of Chemistry  
Yale University

## Rich *E. coli* Media

### Recommended Testing Protocol for BioExpress 1000, Celtone Media, and Spectra 9

All necessary substrates for optimal growth and protein expression are provided in the media. Since bacterial cell growth and recombinant protein expression can be strain specific, this is a general protocol which may need to be modified depending on the specific bacterial strain used.

#### Experiment 1: Growth Curve Study

##### Day 1

1. Beginning with a freshly grown agar plate, choose an isolated colony of bacteria and inoculate a 2 mL culture of Luria broth (LB) (or other standard media) containing the appropriate antibiotic.
2. Shake (or rotate in a roller drum) the culture tube overnight at the appropriate temperature.

##### Day 2

1. In the early morning, the 2 mL culture should be dense ( $OD_{600} \geq 4$ ). Inoculate a control media (e.g. LB) and the labeled media (both containing the appropriate antibiotic) with a 1:100 dilution of the overnight culture.
2. Shake (or rotate) the cultures at the appropriate temperature and collect  $OD_{600}$  data at the following time points ( $T_{\text{hours}}$ ):  $T_0$ ,  $T_2$ ,  $T_3$ ,  $T_4$ ,  $T_5$ ,  $T_6$ ,  $T_7$ ,  $T_8$ , and approximately  $T_{24}$  (or overnight).

#### Experiment 2: Expression Test

##### Day 1

1. Beginning with a freshly grown agar plate, choose an isolated colony of bacteria and inoculate a 2 mL culture of LB (or other standard media) containing the appropriate antibiotic.
2. Shake (or rotate in a roller drum) the culture tube overnight at the appropriate temperature using identical conditions as were used for the growth curve study.

##### Day 2

1. In the early morning check the  $OD_{600}$  of the 2 mL culture (should be  $\geq 4$ ). Inoculate a control media (e.g. LB) and the labeled media (both containing the appropriate antibiotic) with a 1:100 dilution of the overnight culture. Depending on the number of induction time points to be analyzed, a small shake flask may be required (instead of a test tube) to grow a large-enough expression culture.
2. For rich growth media, induce expression of the recombinant protein when the culture reaches an  $OD_{600}$  of 0.6 to 0.8 ( $I_0$ ). At the time of induction remove a 0.5 mL (or 1.0 mL) sample, spin down the culture, and after carefully removing the supernatant, freeze the cell pellet at  $-20^\circ\text{C}$ .
3. Remove and process additional culture samples at the following time points ( $I_{\text{hours}}$ ):  $I_2$ ,  $I_4$ ,  $I_6$ ,  $I_{24}$ .

These cell pellets should then be used for SDS PAGE gel analysis or enzyme activity assays to compare protein expression and determine the optimum time of induction for the specific recombinant protein being produced.

## Helpful Hints

- In general, good aeration is vital to optimal growth and expression. This requires the use of larger (18-25 mm diameter) culture tubes or small shake flasks (with baffles, if available) to allow for maximum aeration. Culture volume should be kept to 20-25% of flask volume.
- Use freshly streaked agar plates to inoculate the growth and expression cultures.
- Cells grow approximately half as fast in deuterated media than in protonated media and also require longer induction times to achieve maximum expression.

## Yeast Media and Reagents

The overexpression of protein in yeast cells represents a powerful expression system for the source of properly folded and functional eukaryotic protein. Please see CIL Application Note 26 at [isotope.com](http://isotope.com) for additional information regarding yeast as a viable expression system to produce isotope-enriched protein for NMR investigations.

Silantes® Yeast-OD2 (rich growth media for yeast) are ready-to-use formulations. The problem of optimizing standard minimal media containing glucose or methanol as a sole carbon source does not arise when using Yeast-OD2 (available in a 1 L packaged size). For yeast that grows well in minimal media (e.g. *Pichia*), CIL offers three different single- and double-labeled carbon sources, as well as in  $^{15}\text{N}$  ammonium salts.

The use of  $\alpha$ -ketobutyrate (CDLM-7318) to label the  $\delta^1$ -methyl of isoleucine has recently been reported in *P. pastoris*<sup>1</sup> which opens up a new avenue of research related to the expression of isotope labeling of eukaryotic proteins.

Please see CIL Application Note 26 at [isotope.com](http://isotope.com) for additional information regarding the use of methanol, glucose, and ammonium salts for use in yeast.

## Yeast Media and Reagents

Catalog No.	Description
CGM-4020-SL-C	Yeast-OD2 ( $^{13}\text{C}$ , 98%)
CGM-4020-SL-N	Yeast-OD2 ( $^{15}\text{N}$ , 98%)
CGM-4020-SL-CN	Yeast-OD2 ( $^{13}\text{C}$ , 98%; $^{15}\text{N}$ , 98%)
CGM-4020-SL-U-S	Yeast-OD2 (unlabeled)
NLM-467	Ammonium chloride ( $^{15}\text{N}$ , 99%)
NLM-713	Ammonium sulfate ( $^{15}\text{N}_2$ , 99%)
CLM-1396	D-Glucose ( $^{13}\text{C}_6$ , 99%)
DLM-2062	D-Glucose ( $\text{D}_7$ , 97-98%)
CDLM-3813	D-Glucose ( $^{13}\text{C}_6$ , 99%; $\text{D}_7$ , 97-98%)
CLM-1510	Glycerol ( $^{13}\text{C}_3$ , 99%)
DLM-558	Glycerol ( $\text{D}_8$ , 99%)
CDLM-7745	Glycerol ( $^{13}\text{C}_3$ , 99%; $\text{D}_8$ , 98%) CP 95%
CDLM-7318	$\alpha$ -Ketobutyric acid, sodium salt (methyl- $^{13}\text{C}$ , 99%; 3,3- $\text{D}_2$ , 98%)
CLM-359	Methanol ( $^{13}\text{C}$ , 99%)
CDLM-1035	Methanol ( $^{13}\text{C}$ , 99%; $\text{D}_3$ , 98%)

### Reference

- Clark, L.; Zahm, J.A.; Ali, R.; et al. **2015**. Methyl labeling and TROSY NMR spectroscopy of proteins expressed in the eukaryote *Pichia pastoris*. *J Biomol NMR*, 62(3), 239-245.

## *Pichia pastoris* as a Eukaryotic Protein Isotope-Labeling System

Investigator  
Spotlight

Leonid S. Brown, PhD, Professor, and Vladimir Ladizhansky, PhD, Professor

Department of Physics, University of Guelph, Ontario Canada

The development of new systems for recombinant expression of isotopically labeled proteins is of significant interest to NMR spectroscopists. While many prokaryotic proteins can readily be over-expressed in *E. coli* and yield functional samples, over-expression of eukaryotic, and, more specifically, medically important mammalian proteins, often requires a different, nonprokaryotic, host.<sup>1-3</sup> Yeast is an alternative choice of eukaryotic expression system that has attracted considerable attention in recent years. Isotopic labeling of proteins in yeast for solution NMR studies began about 25 years ago.<sup>4</sup> Yeast is currently a second most popular expression system after *E. coli*, and seems to offer the best of the two worlds. These systems have the distinct advantages of low labeling costs, high expression yields, ease of genetic manipulation, ability to grow on deuterated media, and speeds of expression close to those in *E. coli*. At the same time, yeast expression allows native folding and an array of post-translational modifications, such as proteolytic truncation, formation of disulfide bonds, glycosylation, phosphorylation, and acylation, typical for eukaryotic cell cultures. Finally, the ability for secreted expression avoids problems with toxicity of the expressed proteins.<sup>1</sup>

### Yeast is an alternative choice of eukaryotic expression system that has attracted considerable attention in recent years.

Among several yeast species used for isotope labeling of proteins, the methylotrophic yeast *Pichia pastoris* accounts for the lion's share of produced structures (about 40 unique proteins).<sup>5</sup> Expression in *Pichia pastoris* usually results in higher expression yields (especially in fermenters) and more native patterns of glycosylation than in other yeast species.<sup>6</sup> Thus, *Pichia pastoris* is the yeast expression system of choice for today's NMR studies of eukaryotic proteins. Another popular choice, *Saccharomyces cerevisiae*, comes in at a distant second. So far, it has yielded nine unique protein structures, with no structures published in the last eight years.<sup>5</sup> Yet another yeast species, *Kluyveromyces lactis*, was recently evaluated for labeled protein expression with promising results.<sup>1</sup>

The uniform  $^{13}\text{C}/^{15}\text{N}$  isotopic labeling in *Pichia pastoris* is very straightforward and follows established protocols, both for shaker incubators and fermenters.<sup>6-8</sup> A large variety of strains and vectors

## *Pichia pastoris* as a Eukaryotic Protein Isotope-Labeling System (continued)

designed for both secreted and nonsecreted expression is available from Invitrogen.<sup>9</sup> Among the highlights are the availability of protease-deficient strains, a number of vectors designed for efficient protein secretion (or membrane-targeting), protein tagging, and selection for multicopy integration transformants. The protocols for the removal of covalently and noncovalently bound sugars are available as well.<sup>10</sup> *Pichia pastoris* grows well on minimal media, where <sup>15</sup>N can be supplied in the form of ammonium salts, and two different <sup>13</sup>C sources are employed in the preinduction and the postinduction phase. As protein expression is induced by methanol and is conducted under the strong alcohol oxidase promoter, <sup>13</sup>C-labeled methanol is the main carbon source in the protein expression phase. Prior to the induction, <sup>13</sup>C-labeled glycerol or glucose should be employed.<sup>6</sup> Economical protocols to minimize the use of those labeled precursors have been developed.<sup>8</sup> Yields of hundreds of milligrams of soluble secreted proteins can be achieved in fermentors at extremely high cell densities. Even flask cultures can produce tens of milligrams of doubly isotopically labeled proteins per liter of culture.<sup>7</sup> Important for NMR applications, a number of deuteration protocols is available,

both for efficient backbone deuteration using protonated carbon sources in D<sub>2</sub>O, as well as for full deuteration.<sup>11,12</sup> Finally, procedures for isotopic labeling of selected amino acid types (Cys, Leu, Lys, and Met) have been published, and *Pichia* strains for labeling aromatic sidechains have been developed.<sup>13,14</sup>

A number of interesting structures of proteins expressed in *Pichia pastoris* have been solved by solution NMR spectroscopy in the last two decades, including the Man-6-P receptor diester recognition domain, cofactor-active fragment of thrombomodulin, collagen-binding domain of discoidin domain receptor 2, and many others.<sup>5</sup> One can expect that in the near future yeast expression systems will emerge as a very valuable tool in the NMR studies of eukaryotic membrane proteins, which are extremely hard to express functionally in bacterial systems and usually too expensive to produce in cell cultures. Multiple successful trials of functional expression of G-protein coupled receptors (GPCRs) and other membrane proteins in *Pichia pastoris* on a milligram scale<sup>15-17</sup> give us optimism on the future of yeast both for solution and solid-state NMR spectroscopy.

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## Insect Cell Media

The baculovirus expression vector system (BEVS), first introduced in the mid-1980s, has grown to become the most versatile and widely used eukaryotic vector system employed for the expression of recombinant proteins in cultured insect cells. The BEVS is based on the infection of insect cells with recombinant baculovirus (BV) carrying the gene of interest with the subsequent expression of the corresponding recombinant protein by the insect cells. The most popular insect cell lines used in conjunction with the BEVS are Sf9 (*Spodoptera frugiperda*) and High Five™ (*Trichopulsia ni*).

CIL is pleased to offer BioExpress 2000, a rich growth media for culturing insect cells. The use of BioExpress 2000 in the uniform <sup>15</sup>N and selective amino acid-type labeling of the catalytic domain of Abl kinase is described in CIL Application Note 14 (see isotope.com). The use of Experimental Design in the optimization of protein yield using BioExpress 2000 is exemplified in CIL Application Note 20 (see isotope.com).

BioExpress 2000 is packaged as two components: a solid powder (a proprietary blend of inorganic salts, carbohydrates, and labeled amino acids) and a liquid component (fatty acid solution). Selective amino acid-type labeling is possible with BioExpress 2000 because the amino acid content is chemically defined.

“My research group develops solid-state NMR methods for protein structure determination, requiring large quantities of <sup>13</sup>C, <sup>15</sup>N-labeled samples produced in-house. We have been very pleased with CIL products for isotopically labeled protein expression, including glucose, glycerol, ammonium chloride, BioExpress, and specifically labeled amino acids. Using CIL products, we routinely obtain several milligrams of labeled membrane proteins per liter of *E. coli* and tens to hundreds of milligrams of water-soluble proteins. We especially appreciate the competitive pricing, outstanding quality control, friendly customer service, and timely delivery of a wide range of products in stock that CIL provides. It's reassuring to know that CIL values its customers and never compromises product quality.”

Chad Rienstra, PhD  
Department of Chemistry  
University of Illinois

## Insect Cell Media

Catalog No.	Description
CGM-2000-N	BioExpress 2000 ( <sup>15</sup> N, 98%)
CGM-2000-N-S	BioExpress 2000 ( <sup>15</sup> N, 98%) reconstitutes to 200 mL
CGM-2000-CN	BioExpress 2000 ( <sup>13</sup> C, 98%; <sup>15</sup> N, 98%)
CGM-2000-U	BioExpress 2000 (unlabeled)
CGM-2000-U-S	BioExpress 2000 (unlabeled) reconstitutes to 200 mL
CGM-2000-CUSTOM	BioExpress 2000 (labeled amino acids specified by customer)

Please inquire for custom media.

### Protocol for Uniform Isotope Labeling of Proteins with BV-infected Sf9 Cells

1. Prior to performing isotope labeling of a protein, optimize culture and BV-infection conditions in unlabeled medium (e.g. BioExpress 2000-U) for expression of the protein.
2. Several 100 mL cultures of Sf9 cells adapted to growth in serum-free medium SF900 II in 500 mL Erlenmeyer flasks are cultivated for 3 days at 27°C, shaken at 90 rpm.
3. Prepare the uniform isotope-labeling medium (e.g. BioExpress 2000-CN) according to CIL's instructions. It can be stored, filter-sterilized for several months at 4°C in the dark without loss of capacity for protein expression. Requires warming up to 28°C before use.
4. When the final cell density of the culture has reached  $\sim 1.5 \times 10^6$  c/mL (~3 days), sterile centrifuge the cells at 400 G for 20 minutes at 20°C.
5. Resuspend the pelleted cells in 100 mL portions in labeled medium (e.g. BioExpress 2000-CN) and transfer to fresh 500 mL Erlenmeyer flasks.
6. Add the recombinant baculovirus of a titer of  $0.5\text{--}2 \times 10^8$  pfu/mL to a MOI=1-2, according to optimized conditions.
7. The 100 mL cultures of BV-infected Sf9 cells are grown for 3 days in labeled medium post infection at 27°C, shaken at 90 rpm.
8. Harvest the cells expressing the labeled recombinant protein by centrifugation (400 G, 20 minutes. at 20°C); resuspend the pelleted cells in PBS, pH 6.2 with protease inhibitor mix (Complete™, Roche) followed by a second centrifugation in 50 mL plastic tubes under conditions as above. Store the pelleted cells at -80°C.
9. Isolate and purify the recombinant protein according to protocols generated for the unlabeled protein. MS and NMR analysis are carried out for proteins labeled in Sf9.

For more information, see CIL Application Note 14 at isotope.com.



## Mammalian Cell Media

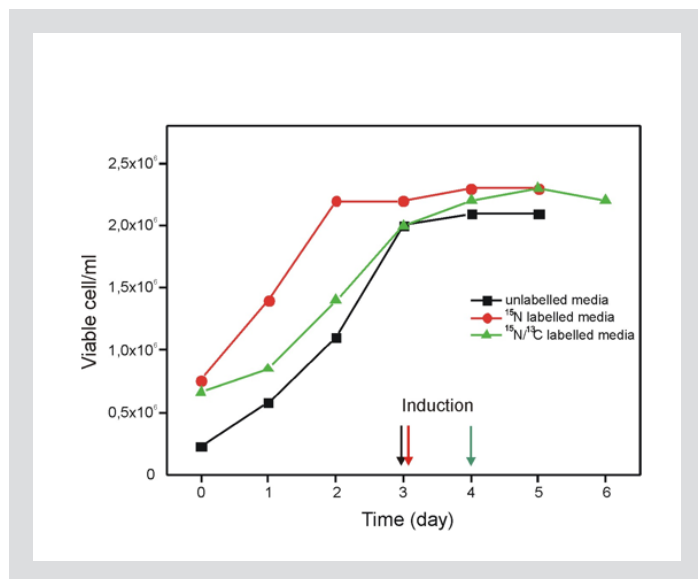
There is continued interest in obtaining labeled recombinant protein from mammalian cells, because eukaryotic protein expressed in mammalian cells has the greatest probability of being properly folded and functional.

CIL offers the only commercially available labeled mammalian media intended for the production of labeled protein with yields suitable for NMR studies. Similar growth characteristics have been obtained using BioExpress 6000 compared to DMEM (Dulbecco's modified Eagle's medium). The amino acid content in BioExpress 6000 is chemically defined so many different custom labeling strategies may be realized. For example, Harald Schwalbe, Karla Werner, and Judith Klein-Seetharaman at Goethe University have used BioExpress 6000 to express rhodopsin from HEK293 cells that is labeled at the Gly, Lys, Leu, Gln, Ser, Thr, Val, and Trp residues with either  $^{15}\text{N}$  or  $^{13}\text{C}/^{15}\text{N}$ .<sup>1</sup> Please see the presented data regarding their application, as well as a growth curve for unlabeled,  $^{15}\text{N}$ -labeled, and  $^{13}\text{C}/^{15}\text{N}$ -labeled BioExpress 6000.

CIL has several different formulations of BioExpress 6000, which is a powdered medium. The CGM-6500 products do not contain sodium pyruvate, allowing users to add a labeled version of sodium pyruvate to the medium, if desired. The CGM-6750 products include HEPES to give extra buffering capacity to the culture medium, which is useful for cells that produce a large amount of lactic acid. The CGM-6800 products do not contain calcium chloride in order to culture nonadherent cells or multicellular spheroids. Unless otherwise noted, the end product reconstitutes to 1000 mL of media.

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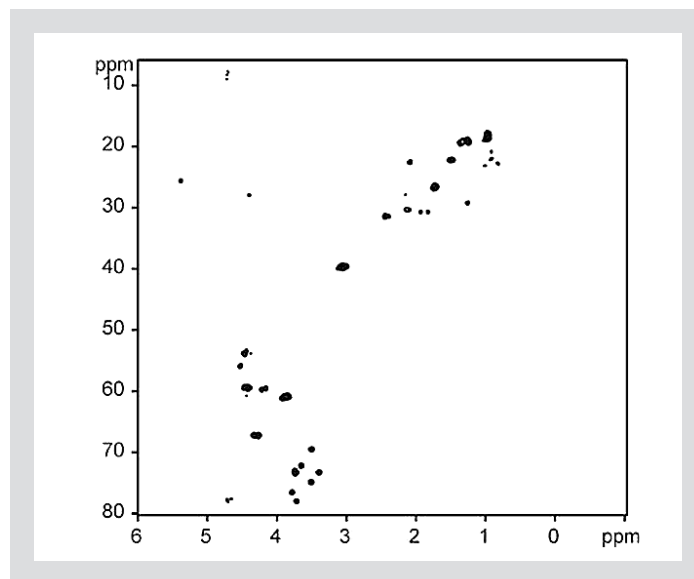


This graph shows the number of viable HEK293 cells per mL of culture for differently labeled CIL mammalian media. Cells are induced on day three and four and harvested two days later. No differences in cell densities are seen so far. Protein yield is approximately 2.2 mg/L cell culture in all cases.

## Mammalian Cell Media

Catalog No.	Description
CGM-6000-N	BioExpress 6000 ( $^{15}\text{N}$ , 98%)
CGM-6000-N-S	BioExpress 6000 ( $^{15}\text{N}$ , 98%) reconstitutes to 200 mL
CGM-6000-CN	BioExpress 6000 ( $^{13}\text{C}$ , 98%; $^{15}\text{N}$ , 98%)
CGM-6000-U-S	BioExpress 6000 (unlabeled) reconstitutes to 200 mL
CGM-6000-CUSTOM	BioExpress 6000 (labeled amino acids specified by customer)
CGM-6500-N	BioExpress 6000 without sodium pyruvate ( $^{15}\text{N}$ , 98%)
CGM-6500-CN	BioExpress 6000 without sodium pyruvate ( $^{13}\text{C}$ , 98%; $^{15}\text{N}$ , 98%)
CGM-6500-CUSTOM	BioExpress 6000 without sodium pyruvate (labeled amino acids specified by customer)
CGM-6750-N	BioExpress 6000 with HEPES ( $^{15}\text{N}$ , 98%)
CGM-6750-CN	BioExpress 6000 with HEPES ( $^{13}\text{C}$ , 98%; $^{15}\text{N}$ , 98%)
CGM-6750-CUSTOM	BioExpress 6000 with HEPES (labeled amino acids specified by customer)
CGM-6750-U-0.25	BioExpress 6000 with HEPES (unlabeled) reconstitutes to 200 mL
CGM-6800-N	BioExpress 6000 without $\text{CaCl}_2$ ( $^{15}\text{N}$ , 98%)
CGM-6800-U-S	BioExpress 6000 without $\text{CaCl}_2$ (unlabeled) reconstitutes to 200 mL

Please inquire for custom media.



$^{13}\text{C}$  HSQC spectrum of  $^{13}\text{C}/^{15}\text{N}$ -labeled rhodopsin showing the sharper peaks due to the more flexible region of the spectrum.

## Cell-Free Production of Stable Isotope-Labeled Proteins

**Investigator  
Spotlight****Masatsune Kainosho, PhD**

Tokyo Metropolitan University, Hachioji, Tokyo and Nagoya University, Nagoya, Japan

Nuclear magnetic resonance (NMR) spectroscopy is used for various purposes in protein science, such as structural biology and drug development. During the last two decades, many of the long-standing methodological difficulties of protein NMR spectroscopy, such as molecular size and sensitivity limitations, have been successfully addressed. For example, using the SAIL (stereo-array isotope labeling) method, it is now routinely possible to determine the high-quality structures of proteins as large as 40-50 kDa, as easily as small proteins.<sup>1</sup> These technological breakthroughs emerged by the synergic development of spectroscopic methodologies and preparative methods of protein samples optimized for collecting the necessary NMR parameters in efficient and accurate manners.

A crucial issue was the development of appropriate protein expression systems, to enable NMR spectroscopists to prepare isotope-labeled protein samples in their own laboratories. It is especially important when using sophisticated stable isotope-aided NMR approaches, such as the SAIL method, to choose a method that efficiently incorporates the expensive labeled amino acids into the targeted proteins without serious metabolic scrambling. This is where the cell-free protein expression systems have made a major contribution. The cell-free expression systems utilize the extracts of various living cells, which contain all of the cellular components relevant for protein synthesis. One can choose the most appropriate host cells, which can be micro-organism, plant or mammalian cells, depending on the type of protein one needs to express.

### The cell-free expression systems utilize the extracts of various living cells, which contain all of the cellular components relevant for protein synthesis.

The cell-free protein expression system actually has a long history, dating back to the initial successful trial to express a protein *in vitro* using an *E. coli* cell extract in the 1960s, as described in the literature.<sup>2</sup> In those early days, the protein production stopped soon after the expression got started, and therefore it was not possible

to prepare the amounts of protein that are required for an NMR or X-ray structural analysis. During the last few decades, however, the expression level has been enormously improved by several key modifications.<sup>3</sup> Now, cell-free protein synthesis methods are commonly used as important alternatives to cellular protein expression systems, for a wide variety of functional and structural studies.<sup>4,5,6</sup> Although most of the current NMR investigations employing cell-free protein production utilize the *E. coli* cell-free extract, it should be noted that the wheat germ extract seems to be quite useful to express “difficult” proteins, such as mammalian proteins or large protein complexes.<sup>7</sup> In addition to the methods using cell extracts, there is a completely different approach that employs a reconstituted protein expression system: the “PURE system.” Since the PURE system is exclusively composed of the purified components necessary for protein synthesis, it will open various new possibilities that cannot be realized by any other methods.<sup>8</sup>

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## Cell-Free Protein Expression

CIL offers a wide variety of products for cell-free protein expression. Cell-free protein expression methods offer several advantages over expression using *E. coli* or other *in vivo* expression systems. These advantages include increased speed, ability to express toxic proteins, ease of amino acid type selective labeling, and an open system that allows cofactors, chaperones, redox molecules, and detergents to be easily be added to the expression system. Cell-free methods also allow co-expression of multiple proteins and are amenable to automation.

CIL is pleased to distribute a wide range of products from CellFree Sciences. CIL also offers algal-derived amino acid mixes and conveniently packaged sizes of individual crystalline amino acids.

Profiles for 16 Amino Acid Mixture (16 AA)		Profiles for 20 Amino Acid Mixture (20 AA)	
Approximate percentages, subject to lot-to-lot variability.			
L-Alanine	7%	L-Alanine	6%
L-Arginine	7%	L-Arginine	6%
L-Aspartic acid	10%	L-Asparagine	5%
L-Glutamic acid	10%	L-Aspartic acid	8%
Glycine	6%	L-Cysteine	3%
L-Histidine	2%	L-Glutamic acid	9%
L-Isoleucine	4%	L-Glutamine	5%
L-Leucine	10%	Glycine	5%
L-Lysine	14%	L-Histidine	1%
L-Methionine	1%	L-Isoleucine	3%
L-Phenylalanine	4%	L-Leucine	9%
L-Proline	7%	L-Lysine	12%
L-Serine	4%	L-Methionine	1%
L-Threonine	5%	L-Phenylalanine	4%
L-Tyrosine	4%	L-Proline	5%
L-Valine	5%	L-Serine	4%
		L-Threonine	4%
		L-Tryptophan	3%
		L-Tyrosine	3%
		L-Valine	4%

## Amino Acid Mixes for Cell-Free Protein Expression

Catalog No.	Description
CLM-1548	Algal amino acid mixture (16AA) ( <sup>13</sup> C, 97-99%)
DLM-2082	Algal amino acid mixture (16AA) (D, 98%)
NLM-2161	Algal amino acid mixture (16AA) ( <sup>15</sup> N, 98%)
CNLM-452	Algal amino acid mixture (16AA) ( <sup>13</sup> C, 97-99%; <sup>15</sup> N, 97-99%)
DNLM-819	Algal amino acid mixture (16AA) (D, 98%; <sup>15</sup> N, 98%)
CDNLM-2496	Algal amino acid mixture (16AA) ( <sup>13</sup> C, 97-99%; D, 97-99%; <sup>15</sup> N, 97-99%)
ULM-2314	Algal amino acid mixture (16AA) (unlabeled)
DLM-6819	"Cell free" amino acid mix (20AA) (D, 98%)
NLM-6695	"Cell free" amino acid mix (20AA) ( <sup>15</sup> N, 96-98%)
CNLM-6696	"Cell free" amino acid mix (20AA) ( <sup>13</sup> C, 97-99%; <sup>15</sup> N, 97-99%)
DNLM-6818	"Cell free" amino acid mix (20AA) (D, 98%; <sup>15</sup> N, 98%)
CDNLM-6784	"Cell free" amino acid mix (20AA) ( <sup>13</sup> C, 97-99%; <sup>15</sup> N, 97-99%; D, 97-99%)
ULM-7891	"Cell free" amino acid mix (20AA) (unlabeled)

► **25 mg and 50 mg packaged sizes are available for all CIL amino acids. Experience the convenience and flexibility of using individually packaged crystalline amino acids with your cell-free protein expression system.\* See page 28 for a complete listing of CIL's free amino acids.**

\*CIL does not provide protocols in formulation of amino acid mixtures, as the formulations may vary depending on application and reaction scale. The pH should be checked prior to use for first-time amino acid formulations.

“We have been a loyal customer of CIL for stable isotope-labeled products for our research in the study of proteins. CIL has provided reliable, in-stock, high-quality products for our lab. We have enjoyed a nice working relationship with CIL over the years.”

Ravi Pratap Barnwal, PhD  
UGC Assistant Professor  
Department of Biophysics  
Panjab University

## Cell-Free Protein Expression

### CellFree Sciences (CFS) Products and Kits for Cell-Free Protein Expression



Producing proteins at will, often a bottleneck in postgenome studies, has become a reality with the advent of the robust wheat germ cell-free protein expression system. CellFree Sciences' ENDEXT® wheat germ cell-free system permits both high throughput protein screening and microgram- to milligram-scale protein production overnight. Protein synthesis protocols for the ENDEXT system have been optimized for a wide range of proteins. They have also been incorporated into robotic protein synthesizers, versatile Protomist® DT II and mass-producing Protomist XE. Being eukaryotic and free from physiological constraints that hamper *in vivo* systems, the wheat germ cell-free system allows synthesis, with or without additives, of a broad spectrum of protein and protein complexes ranging from 10 kDa to 360 kDa in well-folded and

soluble forms. Most importantly, labeled protein samples can be easily produced for NMR studies when labeled amino acids are used.

CIL is pleased to offer a range of CFS products and kits. Please contact CFS directly ([tech-sales@cfsciences.com](mailto:tech-sales@cfsciences.com)) if you would like to use CFS' lab services to prepare a pEU plasmid with your target gene sequence, characterize the yield and solubility of your expressed protein, or produce a prescribed amount of protein using the wheat germ cell-free system.

ENDEXT® is a registered trademark of CellFree Sciences.  
Protomist® is a registered trademark of Emerald BioSystems.  
CIL is a distributor of the above-referenced CFS products in the US and Europe.

Catalog No.	Product Name	Amount	Description
<b>Expression Vectors for Use in Wheat Germ Cell-Free Protein Expression System</b>			
CFS-PEU-v1.0	pEU Vector Set	19 vectors	Vector set for expression of proteins in the wheat germ system: 19 different vectors to work with His-, GST-, and FLAG-tag, untagged proteins. Set includes positive controls and vector for FLEXIQuant method.
<b>Coupled Cell-Free in Vitro Transcription and Translation Reactions for Protein Research Applications</b>			
CFS-EDX-ONE	Premium ONE Expression Kit	24 reactions	Coupled <i>in vitro</i> transcription and translation reaction format for rapid protein expression experiments enabling protein analysis by different detection methods such as SDS-PAGE, Western Blot Analysis, or working with labeled proteins. Kit uses bilayer reaction on 55 µL scale.
<b>Linked Cell-Free Protein Expression Reactions for Protein Research Applications (Bilayer Format)</b>			
<i>Core kits with individual reagents for linked cell-free transcription and translation reactions on different reaction scales</i>			
CFS-C7	WEPRO7240 Core Kit	1 mL extract	SMALL reaction scale of 227 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions).
CFS-C7G	WEPRO7240G Core Kit	1 mL extract	SMALL reaction scale of 227 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions). Kit uses precleared extract intended for preparing GST-fusion proteins.
CFS-C7H	WEPRO7240H Core Kit	1 mL extract	SMALL reaction scale of 227 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions). Kit uses precleared extract intended for preparing His-tagged fusion proteins.
CFS-C8	WEPRO8240 Core Kit	1 mL extract	WEPRO8240 is WEPRO7240 without amino acids to enable protein labeling reactions. SMALL reaction scale of 240 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions).
CFS-C8G	WEPRO8240G Core Kit	1 mL extract	WEPRO8240G is WEPRO7240G without amino acids to enable protein labeling reactions. SMALL reaction scale of 240 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions). Kit uses precleared extract intended for preparing GST-fusion proteins.
CFS-C8H	WEPRO8240H Core Kit	1 mL extract	WEPRO8240H is WEPRO7240H without amino acids to enable protein labeling reactions. SMALL reaction scale of 240 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions). Kit uses precleared extract intended for preparing His-tagged fusion proteins.

## Cell-Free Protein Expression

Catalog No.	Product Name	Amount	Description
<b>Linked Cell-Free Protein Expression Reactions for Protein Research Applications (Bilayer Format) <i>continued</i></b>			
<i>Premixed reagents for linked cell-free transcription and translation reaction on 227 µL scale</i>			
CFS-EDX-PLUS	Premium PLUS Expression Kit	8 reactions	Transcription and translation reagents are premixed for easy handling. Kit uses bilayer reaction on 227 µL scale. This kit contain an expression vector, a positive control, and a PCR primer set.
CFS-PRK-S16	Protein Research Kit S16	16 reactions	Transcription and translation reagents are premixed for easy handling. Kit uses bilayer reaction on 227 µL scale.
CFS-PRK-G16	Protein Research Kit G16	16 reactions	Premixed transcription and translation reagents for GST-fusion protein expression. Kit uses bilayer reaction on 227 µL scale.
CFS-PRK-H16	Protein Research Kit H16	16 reactions	Premixed transcription and translation reagents for His-fusion protein expression. Kit uses bilayer reaction on 227 µL scale.
<b>Linked Cell-Free Protein Expression Reactions for Large Protein Needs in Research/Product Development/Production (Dialysis Format)</b>			
CFS-D7	WEPRO7240 Dialysis Kit	1 mL extract	Dialysis kit provides reagents to perform dialysis-driven expression reactions on a 50 µL small-scale (up to 100 reactions) or preparative 3 mL scale (up to 2 reactions).
CFS-D7G	WEPRO7240G Dialysis Kit	1 mL extract	Dialysis kit provides reagents to perform dialysis-driven expression reactions on a 50 µL small-scale (up to 100 reactions) or preparative 3 mL scale (up to 2 reactions). Kit uses precleared extract intended for preparing GST-fusion proteins.
CFS-D7H	WEPRO7240H Dialysis Kit	1 mL extract	Dialysis kit provides reagents to perform dialysis-driven expression reactions on a 50 µL small-scale (up to 100 reactions) or preparative 3 mL scale (up to 2 reactions). Kit uses precleared extract intended for preparing His-tagged fusion proteins.
<b>Special Applications</b>			
<i>Protein labeling for protein mass spectrometry</i>			
CFS-FLEX-MS	FLEXIQuant PLUS Expression Kit	8 reactions	Protein synthesis kit with <sup>13</sup> C/ <sup>15</sup> N labeled lysine and arginine for MRM/SRM mass spectrometry. Kit uses bilayer reaction on 227 µL scale. This kit provides expression vector and peptide to conduct FLEXIQuant experiments for using the FLEX-tag for quantifying the reference standard.
CFS-FLEX-PEP	FLEX-Tag Peptide	8 vials	Lyophilized, synthetic FLEX-tag peptide (TENLYFQGDISR); >95% peptide purity, 1 nmol dried peptide per vial.
CFS-PRK-MS	Protein Research Kit for MS	16 reactions	Protein synthesis kit with <sup>13</sup> C/ <sup>15</sup> N labeled lysine and arginine for MRM/SRM mass spectrometry. Kit uses bilayer reaction on 227 µL scale.
CFS-C8MS	WEPRO8240 MS Core Kit	1 mL extract	Protein synthesis kit with <sup>13</sup> C/ <sup>15</sup> N labeled lysine and arginine for MRM/SRM mass spectrometry. SMALL reaction scale of 240 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions).
CFS-C8GMS	WEPRO8240G MS Core Kit	1 mL extract	Protein synthesis kit with <sup>13</sup> C/ <sup>15</sup> N labeled lysine and arginine for MRM/SRM mass spectrometry. SMALL reaction scale of 240 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions). Kit uses precleared extract intended for preparing GST-fusion proteins.
CFS-C8HMS	WEPRO8240H MS Core Kit	1 mL extract	Protein synthesis kit with <sup>13</sup> C/ <sup>15</sup> N labeled lysine and arginine for MRM/SRM mass spectrometry. SMALL reaction scale of 240 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions). Kit uses precleared extract intended for preparing His-tagged fusion proteins.

Continued ►



## Cell-Free Protein Expression

Catalog No.	Product Name	Amount	Description
<b>Special Applications – continued</b>			
<i>Protein labeling for NMR</i>			
CFS-C8N	WEPRO8240 ( <sup>15</sup> N) Core Kit	1 mL extract	SMALL reaction scale of 240 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions). The translation buffer contains stable isotope <sup>15</sup> N-labeled amino acids.
CFS-C8GN	WEPRO8240G ( <sup>15</sup> N) Core Kit	1 mL extract	SMALL reaction scale of 240 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions). Kit uses precleared extract intended for preparing GST-fusion proteins. The translation buffer contains stable isotope <sup>15</sup> N-labeled amino acids.
CFS-C8HN	WEPRO8240H ( <sup>15</sup> N) Core Kit	1 mL extract	SMALL reaction scale of 240 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions). Kit uses precleared extract intended for preparing His-tagged fusion proteins. The translation buffer contains stable isotope <sup>15</sup> N-labeled amino acids.
CFS-C8CN	WEPRO8240 ( <sup>13</sup> C/ <sup>15</sup> N) Core Kit	1 mL extract	SMALL reaction scale of 240 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions). The translation buffer contains stable isotope <sup>13</sup> C- and <sup>15</sup> N-labeled amino acids.
CFS-C8GCN	WEPRO8240G ( <sup>13</sup> C/ <sup>15</sup> N) Core Kit	1 mL extract	SMALL reaction scale of 240 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions). Kit uses precleared extract intended for preparing GST-fusion proteins. The translation buffer contains stable isotope <sup>13</sup> C- and <sup>15</sup> N-labeled amino acids.
CFS-C8HCN	WEPRO8240H ( <sup>13</sup> C/ <sup>15</sup> N) Core Kit	1 mL extract	SMALL reaction scale of 240 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions). Kit uses precleared extract intended for preparing His-tagged fusion proteins. The translation buffer contains stable isotope <sup>13</sup> C- and <sup>15</sup> N-labeled amino acids.
<i>Expression of membrane proteins in proteoliposomes</i>			
CFS-EDX-PLUS-PLE	Proteoliposome Plus Expression Kit	8 reactions	Starter kit for easy expression of membrane proteins on 240 µL bilayer reaction scale. Premixed reagents already contain liposomes for easy preparation of proteoliposome complexes.
CFS-CPLE-BDL	Proteoliposome BD Expression Kit-L	6 reactions	For high-yield expression of membrane proteins on 2.5 mL BD reaction scale for direct formation of proteoliposomes. Additional reagents provided for testing expression vector in absence of liposomes.
CFS-ASL	Asolectin Liposomes (lyophilized)	1 set	Set with 6 vials each holding 10 mg of lyophilized liposomes prepared from asolectin (mixture of phospholipids from soybean).
CFS-ASL-BT	Biotinylated Liposomes (lyophilized)	1 set	1 vial holding 10 mg of lyophilized liposomes prepared from asolectin (mixture of phospholipids from soybean) and added biotin.
<i>Expression of proteins with disulfide bonds</i>			
CFS-C7-DTT	WEPRO7240-DTT Core Kit	1 mL extract	The reagents in this kit are DTT free. This allows users to reduce DTT concentrations for improved stability of S-S bonds. SMALL reaction scale of 240 µL (up to 100 reactions), a MEDIUM reaction scale of 1.2 mL (up to 20 reactions), and a LARGE reaction scale of 6 mL (up to 4 reactions). <b>Note:</b> reducing the DTT concentration will commonly reduce protein yields in the translation reactions.
<i>Teaching materials</i>			
CFS-EDU-P	Protein Expression Reagent Kit	10 experiments	Introduction into protein expression doing an <i>in vitro</i> transcription and translation experiment. For one course with 20 students including plastic consumables for use at schools.
CFS-EDU-N	Protein Expression Reagent Kit	10 experiments	Introduction into protein expression doing an <i>in vitro</i> transcription and translation experiment. For one course with 20 students. This version does not provide plastic consumables.
CFS-EDU-E	Laboratory Instrument Set	For one course, reusable	Tube rack, floater, orange filter, blue LED light, UV – LED light.

## Cell-Free Protein Expression

Catalog No.	Product Name	Amount	Description
<b>Individual Reagents</b>			
<i>Transcription reagents</i>			
CFS-TSC-ENZ	SP6 RNA Polymerase plus RNase Inhibitor	80,000 units 1 mL	Enzyme for mRNA synthesis and RNase inhibitor
CFS-TSC-ENZ-S	SP6 RNA Polymerase plus RNase Inhibitor	2,400 units 30 µL	Enzyme for mRNA synthesis and RNase inhibitor
CFS-TSC-ENZ-S/2	SP6 RNA polymerase plus RNase Inhibitor	1,200 units 15 µL	Enzyme for mRNA synthesis and RNase inhibitor
CFS-TSC-NTP	NTP Mix	1 mL	25 mM mixture of ATP, UTP, GTP, and CTP
CFS-TSC-5TB-LM	5x Transcription Buffer LM	1 mL	Transcription buffer for WEPRO7240/8240 series
CFS-TB	Transcription Set (additional set)	1 set	Set contains SP6 RNA polymerase (1,200 units/15 µL), RNase inhibitor (15 µL), 25 mM NTPs (120 µL), and 5x transcription buffer LM (240 µL)
<i>Translation reagents</i>			
CFS-WGE-7240	WEPRO7240	1 mL	High-performance wheat germ extract
CFS-WGE-7240G	WEPRO7240G	1 mL	Precleared high-performance wheat germ extract for working with GST-tagged proteins
CFS-WGE-7240H	WEPRO7240H	1 mL	Precleared high-performance wheat germ extract for working with His-tagged proteins
CFS-WGE-8240	WEPRO8240	1 mL	High-performance wheat germ extract for labeling, containing only traces of amino acids; WEPRO7240 without amino acids
CFS-WGE-8240G	WEPRO8240G	1 mL	High-performance wheat germ extract for labeling, containing only traces of amino acids, preprocessed for GST tag affinity purification; WEPRO7240G without amino acids
CFS-WGE-8240H	WEPRO8240H	1 mL	High-performance wheat germ extract for labeling, containing only traces of amino acids, preprocessed for His tag affinity purification; WEPRO7240H without amino acids
CFS-SUB-SGC	SUB-AMIX SGC	12.5 mL × 4	Translation buffer; to be used with WEPRO7240 series
CFS-SUB	SUB-AMIX SGC SUB Set	7 mL × 4	Translation buffer; to be used with WEPRO7240 series
CFS-SUB-SGC-NA	SUB-AMIX SGC NA	12.5 mL × 4	Translation buffer for labeling; SUB-AMIX SGC without amino acids; to be used with WEPRO8240 series
CFS-SUB-SG	SUB-AMIX SG	12.5 mL × 4	Translation buffer; to be used with WEPRO7240 series on Protomist XE
CFS-SUB-SG-NAA	SUB-AMIX SG NA	12.5 mL × 4	Translation buffer for labeling; SUB-AMIX SG without amino acids; to be used with WEPRO8240 series on Protomist XE
<b>Consumables</b>			
<i>Protomist® DT II</i>			
CFS-DT2-P01-1	Reaction Cups	1 × 6 cups	6 × disposable reaction cups
CFS-DT2-P01-10	Reaction Cups	10 × 6 cups	60 × disposable reaction cups
CFS-DT2-P01-50	Reaction Cups	50 × 6 cups	300 × disposable reaction cups
CFS-ASP-DIP1000V	Robotic Tips (bulk)	2 × 500 tips	1000 × disposable pipetting tips
CFS-ASP-DIP1000V-PT24	Robotic Tips (racked)	24 × 96 tips	24 × ready-to-use disposable pipetting tips in 96-well racks
CFS-DT2-P02-2	Tubes	2 × 500 tubes	1000 × 2 mL tubes
<i>Protomist XE</i>			
CFS-XE-P10	Preassembled Kit	1 set	10 × 10 mL reactions
CFS-XE-P20	Preassembled Kit	1 set	10 × 20, 30, 40 mL reactions
CFS-XE-VFC	Connectors	1 set	24 × Vivaflow connector (needed for 30 and 40 mL reactions)

## Selective Amino Acid-Type Labeling

Selective amino acid-type labeling is used to simplify crowded spectra in order to aid in spectral interpretation or to provide specific probes for dynamic and structural studies. This technique requires the addition of labeled amino acids (~50-250 mg/L) to either minimal or rich growth media prior to protein induction.

The cells will generally utilize the supplemented amino acids for protein synthesis prior to undergoing the *de novo* synthesis of the target amino acids. Please see page 28 for a complete listing of amino acids that may be used with either *in vivo* growth systems or cell-free protein expression methods.

## Selective Isotope-Labeling Methods for Protein Structural Studies

Investigator  
Spotlight

Hanudatta S. Atreya, PhD

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One of the major contributing factors to the rapid advance of biomolecular NMR spectroscopy is the emergence of different isotope-labeling methods. Recent developments in biotechnology have made it easier and economical to introduce  $^{13}\text{C}$ ,  $^{15}\text{N}$  and  $^2\text{H}$  into proteins and nucleic acids. At the same time, there has been an explosion in the number of NMR experiments that utilize such isotope-labeled samples. Thus, a combination of isotopic labeling and multidimensional, multinuclear experiments has significantly expanded the range of problems in structural biology amenable to NMR.

Isotope labeling in proteins can be broadly classified into four categories: uniform, amino acid-type selective, site-specific, and random/fractional labeling. The beginning of systematized isotope labeling in proteins can be traced back to late 60s in the group of Jardetsky and Katz and coworkers.<sup>1,2</sup> Theirs was also one of the first amino acid-type selective-labeling methods involving incorporation of specific protonated amino acids against a deuterated background. In the 80s uniform ( $^{13}\text{C}/^{15}\text{N}$ ) and selective incorporation of  $^{15}\text{N}$ -labeled amino acids against an unlabeled ( $^{12}\text{C}/^{14}\text{N}$ ) background was developed.<sup>3</sup> Subsequently, a variety of labeling methods have emerged (reviewed in [4] and [5] and illustrated in Figure 1).

In addition to uniform ( $^{13}\text{C}/^{15}\text{N}/^2\text{H}$ ) labeling, amino acid-type or site-selective labeling is often pursued as it helps in spectral simplification and provides specific probes for structural and dynamic studies. Selective amino acid-type labeling also aids in sequence-specific resonance assignments by helping to identify resonances which are otherwise buried in the crowded regions of 2D and 3D NMR spectra. However, a disadvantage of this method is the possible mis-incorporation of  $^{15}\text{N}$  label in undesired amino acids (also called as “isotope scrambling”).<sup>3</sup> This happens due to metabolic conversion of one amino acid to another in the biosynthetic pathway of the cell. The problem becomes more severe for amino acids or intermediates higher up in the metabolic pathway such as Asp, Glu and Gln (See Figure 2 showing the biosynthetic pathway in *E. coli*). For those which are end-products in the production pipeline (Ala, Arg, Asn, Cys, His, Ile, Lys, Met, Pro, and Trp), isotope scrambling is minimal, and the remaining (Gly, Phe, Leu, Ser, Thr, Tyr, and Val) have medium to weak interconversion. Isotope scrambling in *E. coli* can be minimized by reducing the activity of the enzyme(s) catalyzing the interconversion or amino acid transfer using either specific (auxotrophic) strains<sup>3</sup> or using enzyme inhibitors.<sup>6</sup> Another alternative is to use cell-free or *in vitro* expression systems which lack these enzymes.<sup>4</sup>

**Selective amino acid-type labeling also aids in sequence-specific resonance assignments by helping to identify resonances which are otherwise buried in the crowded regions of 2D and 3D NMR spectra.**

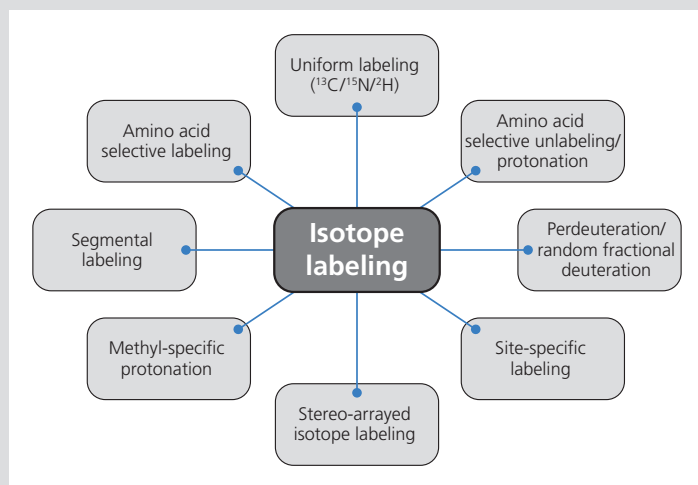


Figure 1. Different isotope-labeling methods.

One drawback of amino acid selective labeling is the expense associated with the use of  $^{13}\text{C}/^{15}\text{N}$  labeled amino acids. A relatively inexpensive method is that of amino acid selective “unlabeling” or reverse labeling. In this method, the host organism is grown on a medium containing the desired unlabeled (*i.e.*,  $^1\text{H}/^{12}\text{C}/^{14}\text{N}$ ) amino acid against a labeled ( $^{13}\text{C}/^{15}\text{N}$ ) background. This is somewhat akin to the selective protonation experiment by Jardetsky<sup>1</sup> and Katz.<sup>2</sup> Reverse labeling was first used by Bax and coworkers<sup>7</sup> and developed further by other groups for different applications.<sup>8,9,10</sup> The problem of isotope scrambling (in this case being the mis-incorporation of  $^{14}\text{N}$ ) remains largely the same as in the selective-labeling approach mentioned above (for a detailed table of possible scrambling of  $^{14}\text{N}$  see reference 10).

## Selective Isotope-Labeling Methods for Protein Structural Studies (continued)

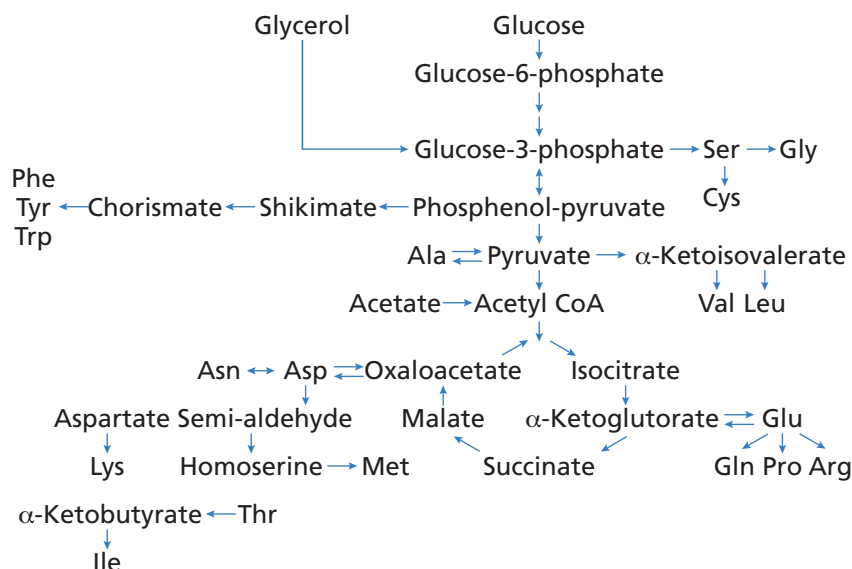


Figure 2. Amino acid biosynthesis in *E. coli*.

In addition to the above, new isotope-labeling methods continue to be developed. Additional methods such as segmental labeling<sup>11</sup> and stereo-arrayed isotope labeling (SAIL)<sup>12</sup> have opened up new

avenues in protein structural studies. The future points toward a combination of different isotope-labeling methods to address challenging and complex problems in structural biology.

### References

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## Amino Acids

CIL offers a comprehensive list of labeled amino acids for use in a variety of NMR investigations. The large number of available labeling schemes maximizes flexibility involved in designing experiments to probe structure, dynamics and binding of biological macromolecules. A number of package sizes are available, including 25 mg and 50 mg, which are convenient for use in cell-free protein expression. Pricing and availability can be found on [isotope.com](http://isotope.com). If a specific size is not listed, custom packaging may be available; please inquire.

Catalog No.	Description	Unit Size
CLM-8755	$\beta$ -Alanine ( $3\text{-}^{13}\text{C}$ , 99%)	Please inquire
CLM-8756	$\beta$ -Alanine ( $^{13}\text{C}_3$ , 99%)	Please inquire
NLM-1656	$\beta$ -Alanine ( $^{15}\text{N}$ , 98%)	0.25 g
CNLM-3440	$\beta$ -Alanine ( $3\text{-}^{13}\text{C}$ , 99%; $^{15}\text{N}$ , 98%)	Please inquire
CNLM-8457	$\beta$ -Alanine ( $1,2\text{-}^{13}\text{C}_2$ , 99%; $^{15}\text{N}$ , 98%)	Please inquire
CNLM-3946	$\beta$ -Alanine ( $^{13}\text{C}_3$ , 98%; $^{15}\text{N}$ , 96-99%)	0.25 g
CLM-116	L-Alanine ( $1\text{-}^{13}\text{C}$ , 99%)	0.5 g, 1 g
CLM-2016	L-Alanine ( $2\text{-}^{13}\text{C}$ , 99%)	0.1 g, 0.25 g, 0.5 g
CLM-117	L-Alanine ( $3\text{-}^{13}\text{C}$ , 99%)	0.5 g, 1 g
CLM-2734	L-Alanine ( $2,3\text{-}^{13}\text{C}_2$ , 99%)	0.25 g, 0.5 g
CLM-2184-H	L-Alanine ( $^{13}\text{C}_3$ , 99%)	0.1 g, 0.25 g, 0.5 g
DLM-3101	L-Alanine ( $2\text{-D}$ , 96-98%)	Please inquire
DLM-248	L-Alanine ( $3,3,3\text{-D}_3$ , 99%)	1 g
DLM-250	L-Alanine ( $2,3,3,3\text{-D}_4$ , 98%)	0.1 g, 1 g
DLM-251	L-Alanine ( $\text{D}_7$ , 98%)	1 g
NLM-454	L-Alanine ( $^{15}\text{N}$ , 98%)	0.5 g, 1 g
CDLM-8649	L-Alanine ( $3\text{-}^{13}\text{C}$ , 99%; $2\text{-D}$ , 96%)	1 g
CDLM-3439	L-Alanine ( $3\text{-}^{13}\text{C}$ , 99%; $3,3,3\text{-D}_3$ , 98%)	Please inquire
CNLM-6993	L-Alanine ( $1\text{-}^{13}\text{C}$ , 99%; $^{15}\text{N}$ , 98%)	0.25 g
CNLM-3594	L-Alanine ( $2\text{-}^{13}\text{C}$ , 99%; $^{15}\text{N}$ , 98%)	0.25 g
CNLM-534-H	L-Alanine ( $^{13}\text{C}_3$ , 99%; $^{15}\text{N}$ , 99%)	0.1 g, 0.25 g, 0.5 g,
DNLM-7178	L-Alanine ( $2,3,3,3\text{-D}_4$ , 98%; $^{15}\text{N}$ , 98%)	0.25 g, 0.5 g
CDNLM-6800	L-Alanine ( $^{13}\text{C}_3$ , 97-99%; $\text{D}_4$ , 97-99%; $^{15}\text{N}$ , 97-99%)	0.25 g
CLM-2070	L-Arginine-HCl (guanido- $^{13}\text{C}$ , 99%)	0.5 g
CLM-1268	L-Arginine-HCl ( $1\text{-}^{13}\text{C}$ , 99%)	0.1 g
CLM-2051	L-Arginine-HCl ( $1,2\text{-}^{13}\text{C}_2$ , 99%)	0.1 g
CLM-2265-H	L-Arginine-HCl ( $^{13}\text{C}_6$ , 99%)	0.05 g, 0.1 g, 0.25 g, 0.5 g, 1 g
DLM-6038	L-Arginine-HCl ( $4,4,5,5\text{-D}_4$ , 94%)	Please inquire
DLM-541	L-Arginine-HCl ( $\text{D}_7$ , 98%)	0.1 g
NLM-1267	L-Arginine-HCl ( $\alpha\text{-}^{15}\text{N}$ , 98%)	Please inquire
NLM-395	L-Arginine-HCl (guanido- $^{15}\text{N}_2$ , 98%)	0.5 g, 1 g
NLM-396	L-Arginine-HCl ( $^{15}\text{N}_4$ , 98%)	0.1 g
CDLM-3789	L-Arginine-HCl ( $5\text{-}^{13}\text{C}$ , 99%; $4,4,5,5\text{-D}_4$ , 95%)	Please inquire
CNLM-7819	L-Arginine-HCl ( $1\text{-}^{13}\text{C}$ , 99%; $\alpha\text{-}^{15}\text{N}$ , 98%)	Please inquire
CNLM-539-H	L-Arginine-HCl ( $^{13}\text{C}_6$ , 99%; $^{15}\text{N}_4$ , 99%)	0.05 g, 0.1 g, 0.25 g, 0.5 g, 1 g
DNLM-7543	L-Arginine-HCl ( $\text{D}_7$ , 98%; $^{15}\text{N}_4$ , 98%)	0.25 g
CDNLM-6801	L-Arginine-HCl ( $^{13}\text{C}_6$ , 97-99%; $\text{D}_7$ , 97-99%; $^{15}\text{N}_4$ , 97-99%)	0.25 g
ULM-8347	L-Arginine-HCl (unlabeled)	0.05 g, 0.1 g
CLM-8699-H	L-Asparagine-H <sub>2</sub> O ( $^{13}\text{C}_4$ , 99%)	0.05 g
DLM-6844	L-Asparagine-H <sub>2</sub> O ( $2,3,3\text{-D}_3$ , 94%)	0.1 g
NLM-120	L-Asparagine-H <sub>2</sub> O (amide- $^{15}\text{N}$ , 98%)	0.25 g, 0.5 g
NLM-3286	L-Asparagine-H <sub>2</sub> O ( $^{15}\text{N}_2$ , 98%)	0.25 g, 0.5 g
CNLM-7818	L-Asparagine-H <sub>2</sub> O ( $1,4\text{-}^{13}\text{C}_2$ , 99%; $\alpha\text{-}^{15}\text{N}$ , 98%)	0.25 g
CNLM-3819-H	L-Asparagine-H <sub>2</sub> O ( $^{13}\text{C}_4$ , 99%; $^{15}\text{N}_2$ , 99%)	0.1 g, 0.25 g, 0.5 g
DNLM-6932	L-Asparagine-H <sub>2</sub> O ( $2,3,3\text{-D}_3$ , 98%; $^{15}\text{N}_2$ , 98%)	0.25 g
CDNLM-6802	L-Asparagine-H <sub>2</sub> O ( $^{13}\text{C}_4$ , 97-99%; $\text{D}_3$ , 97-99%; $^{15}\text{N}_2$ , 97-99%)	0.25 g

Chemical purity (CP) is 98% or greater, unless otherwise specified.

For research use only. Not for use in diagnostic procedures.



Catalog No.	Description	Unit Size
CLM-3616	L-Aspartic acid (1- <sup>13</sup> C, 99%)	Please inquire
CLM-3617	L-Aspartic acid (2- <sup>13</sup> C, 99%)	Please inquire
CLM-627	L-Aspartic acid (3- <sup>13</sup> C, 98-99%)	0.05 g, 0.1 g, 0.25 g
CLM-519	L-Aspartic acid (4- <sup>13</sup> C, 99%) CP 96%	Please inquire
CLM-4455	L-Aspartic acid (1,4- <sup>13</sup> C <sub>2</sub> , 99%)	0.5 g
CLM-1801-H	L-Aspartic acid ( <sup>13</sup> C <sub>4</sub> , 99%)	0.25 g, 0.5 g, 1 g
DLM-546	L-Aspartic acid (2,3,3-D <sub>3</sub> , 98%)	0.1 g, 0.25 g
NLM-718	L-Aspartic acid ( <sup>15</sup> N, 98%)	0.5 g, 1 g
CNLM-7817	L-Aspartic acid (1,4- <sup>13</sup> C <sub>2</sub> , 99%; <sup>15</sup> N, 98%)	0.25 g
CNLM-544-H	L-Aspartic acid ( <sup>13</sup> C <sub>4</sub> , 99%; <sup>15</sup> N, 99%)	0.25 g, 0.5 g, 1 g
DNLM-6931	L-Aspartic acid (2,3,3-D <sub>3</sub> , 98%; <sup>15</sup> N, 98%)	0.25 g, 0.5 g
CDNLM-6803	L-Aspartic acid ( <sup>13</sup> C <sub>4</sub> , 97-99%; D <sub>3</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.25 g
CLM-3852	L-Cysteine (1- <sup>13</sup> C, 99%)	0.5 g
CLM-1868	L-Cysteine (3- <sup>13</sup> C, 99%)	0.25 g
CLM-4320-H	L-Cysteine ( <sup>13</sup> C <sub>3</sub> , 99%)	0.1 g
DLM-769	L-Cysteine (3,3-D <sub>2</sub> , 98%)	0.1 g
DLM-6901	L-Cysteine (2,3,3-D <sub>3</sub> , 98%)	0.1 g
NLM-2295	L-Cysteine ( <sup>15</sup> N, 98%)	0.25 g
CNLM-7815	L-Cysteine (1- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	Please inquire
CNLM-3871-H	L-Cysteine ( <sup>13</sup> C <sub>3</sub> , 99%; <sup>15</sup> N, 99%)	0.1 g, 0.25 g
DNLM-6902	L-Cysteine (2,3,3-D <sub>3</sub> , 98%; <sup>15</sup> N, 98%)	0.25 g
CDNLM-6809	L-Cysteine ( <sup>13</sup> C <sub>3</sub> , 97-99%; D <sub>3</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.25 g
CLM-520	L-Cystine (3,3'- <sup>13</sup> C <sub>2</sub> , 99%)	0.25 g
DLM-9812	L-Cystine (3,3,3',3'-D <sub>4</sub> , 98%)	0.5 g
NLM-3818	L-Cystine ( <sup>15</sup> N <sub>2</sub> , 98%)	0.25 g
CNLM-4244-H	L-Cystine ( <sup>13</sup> C <sub>6</sub> , 99%; <sup>15</sup> N <sub>2</sub> , 99%)	Please inquire
CDNLM-8659	L-Cystine ( <sup>13</sup> C <sub>6</sub> , 98%; D <sub>6</sub> , 98%; <sup>15</sup> N <sub>2</sub> , 98%) CP 95%	Please inquire
CLM-674	L-Glutamic acid (1- <sup>13</sup> C, 99%)	1 g
CLM-2474	L-Glutamic acid (2- <sup>13</sup> C, 99%)	Please inquire
CLM-4742	L-Glutamic acid (3- <sup>13</sup> C, 99%)	Please inquire
CLM-2431	L-Glutamic acid (4- <sup>13</sup> C, 98-99%)	Please inquire
CLM-613	L-Glutamic acid (5- <sup>13</sup> C, 99%)	0.1 g
CLM-2024	L-Glutamic acid (1,2- <sup>13</sup> C <sub>2</sub> , 99%)	0.25 g
CLM-3646	L-Glutamic acid (3,4- <sup>13</sup> C <sub>2</sub> , 99%)	0.25 g
CLM-1800-H	L-Glutamic acid ( <sup>13</sup> C <sub>5</sub> , 99%)	0.25 g, 0.5 g, 1 g
DLM-3725	L-Glutamic acid (2,4,4-D <sub>3</sub> , 97-98%)	0.5 g
DLM-556	L-Glutamic acid (2,3,3,4,4-D <sub>5</sub> , 98%)	0.05 g, 0.1 g
NLM-135	L-Glutamic acid ( <sup>15</sup> N, 98%)	0.5 g, 1 g
CNLM-7812	L-Glutamic acid (1- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	0.25 g
CNLM-554-H	L-Glutamic acid ( <sup>13</sup> C <sub>5</sub> , 99%; <sup>15</sup> N, 99%)	0.25 g, 0.5 g, 1 g
DNLM-6996	L-Glutamic acid (2,3,3,4,4-D <sub>5</sub> , 98%; <sup>15</sup> N, 98%)	0.25 g, 0.5 g
CDNLM-6804	L-Glutamic acid ( <sup>13</sup> C <sub>5</sub> , 97-99%; D <sub>5</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.25 g
OLM-8028	L-Glutamic acid-HCl ( <sup>17</sup> O <sub>4</sub> , ~30%)	Please inquire
CLM-3612	L-Glutamine (1- <sup>13</sup> C, 99%)	1 g
CLM-3613	L-Glutamine (2- <sup>13</sup> C, 99%)	Please inquire
CLM-770	L-Glutamine (4- <sup>13</sup> C, 99%)	Please inquire
CLM-1166	L-Glutamine (5- <sup>13</sup> C, 99%)	0.25 g
CLM-2001	L-Glutamine (1,2- <sup>13</sup> C <sub>2</sub> , 99%)	0.1 g
CLM-3641	L-Glutamine (3,4- <sup>13</sup> C <sub>2</sub> , 99%)	Please inquire
CLM-1822-H	L-Glutamine ( <sup>13</sup> C <sub>5</sub> , 99%)	0.1 g, 0.25 g, 0.5 g
DLM-1826	L-Glutamine (2,3,3,4,4-D <sub>5</sub> , 97%)	0.1 g
NLM-1016	L-Glutamine (α- <sup>15</sup> N, 98%)	0.1 g, 1 g

Chemical purity (CP) is 98% or greater, unless otherwise specified.

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## Amino Acids (continued)

Catalog No.	Description	Unit Size
NLM-557	L-Glutamine (amide- <sup>15</sup> N, 98%)	0.5 g, 1 g
NLM-1328	L-Glutamine ( <sup>15</sup> N <sub>2</sub> , 98%)	0.25 g
CNLM-7813	L-Glutamine (1- <sup>13</sup> C, 99%; α- <sup>15</sup> N, 98%)	Please inquire
CNLM-1275-H	L-Glutamine ( <sup>13</sup> C <sub>5</sub> , 99%; <sup>15</sup> N <sub>2</sub> , 99%)	0.1 g, 0.25 g, 0.5 g
DNLM-6997	L-Glutamine (2,3,3,4,4-D <sub>5</sub> , 97-98%; <sup>15</sup> N <sub>2</sub> , 97-98%)	0.25 g
CDNLM-6805	L-Glutamine ( <sup>13</sup> C <sub>5</sub> , 97-99%; D <sub>5</sub> , 97-99%; <sup>15</sup> N <sub>2</sub> , 97-99%)	0.25 g
CLM-422	Glycine (1- <sup>13</sup> C, 99%)	1 g, 5 g
CLM-136	Glycine (2- <sup>13</sup> C, 99%)	0.5 g, 1 g, 5 g
CLM-1017	Glycine (1,2- <sup>13</sup> C <sub>2</sub> , 97-99%)	0.5 g, 1 g, 5 g
DLM-1674	Glycine (2,2-D <sub>2</sub> , 98%)	5 g
DLM-280	Glycine (D <sub>5</sub> , 98%)	5 g
DLM-280-80	Glycine (D <sub>5</sub> , 80%)	5 g
NLM-202	Glycine ( <sup>15</sup> N, 98%)	1 g, 5 g
CNLM-507	Glycine (1- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	1 g
CNLM-508	Glycine (2- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	0.5 g, 1 g
CNLM-1673-H	Glycine ( <sup>13</sup> C <sub>2</sub> , 99%; <sup>15</sup> N, 99%)	0.25 g, 0.5 g, 1 g
DNLM-6862	Glycine (2,2-D <sub>2</sub> , 98%; <sup>15</sup> N, 98%)	0.25 g, 0.5 g
CDNLM-6799	Glycine ( <sup>13</sup> C <sub>2</sub> , 97-99%; 2,2-D <sub>2</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.25 g
NLM-4649	L-Histidine (ring-ε- <sup>15</sup> N, 98%)	Please inquire
NLM-4457	L-Histidine (ring-π- <sup>15</sup> N, 98%)	Please inquire
NLM-9585	L-Histidine (ring- <sup>15</sup> N <sub>2</sub> , 98%)	Please inquire
CLM-1512	L-Histidine·HCl·H <sub>2</sub> O (ring-2- <sup>13</sup> C, 99%)	0.1 g
CLM-2264	L-Histidine·HCl·H <sub>2</sub> O ( <sup>13</sup> C <sub>6</sub> , 97-99%)	0.05 g, 0.1 g, 0.25 g
DLM-7855	L-Histidine·HCl·H <sub>2</sub> O (ring-2,4-D <sub>2</sub> ; α,β,β-D <sub>3</sub> , 98%)	0.25 g
NLM-2245	L-Histidine·HCl·H <sub>2</sub> O (α- <sup>15</sup> N, 98%)	0.25 g
NLM-846	L-Histidine·HCl·H <sub>2</sub> O (ring-π- <sup>15</sup> N, 98%)	Please inquire
NLM-1513	L-Histidine·HCl·H <sub>2</sub> O ( <sup>15</sup> N <sub>3</sub> , 98%)	0.25 g
CNLM-758	L-Histidine·HCl·H <sub>2</sub> O ( <sup>13</sup> C <sub>6</sub> , 97-99%; <sup>15</sup> N <sub>3</sub> , 97-99%) (<5% D)	0.25 g
DNLM-7366	L-Histidine·HCl·H <sub>2</sub> O (D <sub>5</sub> , 98%; <sup>15</sup> N <sub>3</sub> , 98%)	0.25 g
CDNLM-6806	L-Histidine·HCl·H <sub>2</sub> O ( <sup>13</sup> C <sub>6</sub> , 97-99%; D <sub>5</sub> , 97-99%; <sup>15</sup> N <sub>3</sub> , 97-99%)	0.25 g
CLM-1026	L-Isoleucine (1- <sup>13</sup> C, 99%)	0.5 g, 1 g
CLM-2248-H	L-Isoleucine ( <sup>13</sup> C <sub>6</sub> , 99%)	0.05 g, 0.1 g, 0.25 g
DLM-141	L-Isoleucine (D <sub>10</sub> , 98%)	0.1 g, 0.25 g
NLM-292	L-Isoleucine ( <sup>15</sup> N, 98%)	0.25 g
CNLM-7810	L-Isoleucine (1- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	Please inquire
CNLM-561-H	L-Isoleucine ( <sup>13</sup> C <sub>6</sub> , 99%; <sup>15</sup> N, 99%)	0.05 g, 0.1 g, 0.25 g
DNLM-7325	L-Isoleucine (D <sub>10</sub> , 98%; <sup>15</sup> N, 98%)	0.25 g
CDNLM-6807	L-Isoleucine ( <sup>13</sup> C <sub>6</sub> , 97-99%; D <sub>10</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.25 g
CLM-468	L-Leucine (1- <sup>13</sup> C, 99%)	1 g, 5 g
CLM-2014	L-Leucine (2- <sup>13</sup> C, 99%)	0.5 g, 1 g
CLM-3524	L-Leucine (1,2- <sup>13</sup> C <sub>2</sub> , 99%)	0.25 g
CLM-2262-H	L-Leucine ( <sup>13</sup> C <sub>6</sub> , 99%)	0.05 g, 0.1 g, 0.25 g
DLM-1259	L-Leucine (5,5,5-D <sub>3</sub> , 99%)	1 g, 5 g
DLM-4212	L-Leucine (isopropyl-D <sub>7</sub> , 98%)	1 g
DLM-567	L-Leucine (D <sub>10</sub> , 98%)	0.25 g
NLM-142	L-Leucine ( <sup>15</sup> N, 98%)	0.5 g, 1 g
CNLM-615	L-Leucine (1- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	1 g
CNLM-615-95	L-Leucine (1- <sup>13</sup> C, 99%; <sup>15</sup> N, 93-95%)	1 g
CNLM-3450	L-Leucine (2- <sup>13</sup> C, 99%; <sup>15</sup> N, 95-99%)	0.5 g
CNLM-281-H	L-Leucine ( <sup>13</sup> C <sub>6</sub> , 99%; <sup>15</sup> N, 99%)	0.05 g, 0.1 g, 0.25 g
DNLM-4642	L-Leucine (D <sub>10</sub> , 98%; <sup>15</sup> N, 97%)	0.25 g, 0.5 g
CDNLM-6808	L-Leucine ( <sup>13</sup> C <sub>6</sub> , 97-99%; D <sub>10</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.25 g

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Catalog No.	Description	Unit Size
CLM-653	L-Lysine·2HCl (1- <sup>13</sup> C, 99%)	0.25 g, 0.5 g
CLM-632	L-Lysine·2HCl (6- <sup>13</sup> C, 99%)	0.25 g
CLM-2247-H	L-Lysine·2HCl ( <sup>13</sup> C <sub>6</sub> , 99%)	0.05 g, 0.1 g, 0.25 g, 0.5 g, 1 g
DLM-2640	L-Lysine·2HCl (4,4,5,5-D <sub>4</sub> , 96-98%)	0.25 g, 0.5 g, 1 g
DLM-2641	L-Lysine·2HCl (3,3,4,4,5,5,6,6-D <sub>8</sub> , 98%)	0.25 g
DLM-570	L-Lysine·2HCl (D <sub>9</sub> , 98%)	0.1 g
NLM-143	L-Lysine·2HCl (α- <sup>15</sup> N, 95-99%)	0.25 g, 1 g
NLM-631	L-Lysine·2HCl (ε- <sup>15</sup> N, 98%)	0.5 g
NLM-1554	L-Lysine·2HCl ( <sup>15</sup> N <sub>2</sub> , 98%)	0.1 g
CNLM-7821	L-Lysine·2HCl (1- <sup>13</sup> C, 99%; α- <sup>15</sup> N, 98%)	Please inquire
CNLM-3454	L-Lysine·2HCl (6- <sup>13</sup> C, 99%; ε- <sup>15</sup> N, 98%)	Please inquire
CNLM-291-H	L-Lysine·2HCl ( <sup>13</sup> C <sub>6</sub> , 99%; <sup>15</sup> N <sub>2</sub> , 99%)	0.05 g, 0.1 g, 0.25 g, 0.5 g, 1 g
DNLM-7545	L-Lysine·2HCl (D <sub>9</sub> , 98%; <sup>15</sup> N <sub>2</sub> , 98%)	0.25 g
CDNLM-6810	L-Lysine·2HCl ( <sup>13</sup> C <sub>6</sub> , 97-99%; D <sub>9</sub> , 97-99%; <sup>15</sup> N <sub>2</sub> , 97-99%)	0.25 g
ULM-8766	L-Lysine·2HCl (unlabeled)	0.05 g, 0.1 g
CLM-206	L-Methionine (methyl- <sup>13</sup> C, 99%)	1 g
CLM-3267	L-Methionine (1- <sup>13</sup> C, 99%)	0.25 g, 1 g
CLM-893-H	L-Methionine ( <sup>13</sup> C <sub>5</sub> , 99%)	0.05 g, 0.1 g, 0.25 g
DLM-431	L-Methionine (methyl-D <sub>3</sub> , 98%)	1 g, 5 g
DLM-6797	L-Methionine (2,3,3,4,4-D <sub>5</sub> ; methyl-D <sub>3</sub> , 98%)	0.1 g
NLM-752	L-Methionine ( <sup>15</sup> N, 96-98%)	0.5 g, 1 g
CDLM-760	L-Methionine (1- <sup>13</sup> C, 99%; methyl-D <sub>3</sub> , 98%)	Please inquire
CDLM-9289	L-Methionine (methyl- <sup>13</sup> C, 99%; methyl-D <sub>3</sub> , 98%)	0.25 g, 1 g
CDLM-8885	L-Methionine (methyl- <sup>13</sup> CH <sub>3</sub> , 99%; 2,3,3,4,4-D <sub>5</sub> , 98%)	0.5 g, 1 g
CNLM-7807	L-Methionine (1- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	0.25 g
CNLM-9774	L-Methionine (1,2,3,4- <sup>13</sup> C <sub>4</sub> , 99%; <sup>15</sup> N, 98%)	Please inquire
CNLM-759-H	L-Methionine ( <sup>13</sup> C <sub>5</sub> , 99%; <sup>15</sup> N, 99%)	0.05 g, 0.1 g, 0.25 g
DNLM-7179	L-Methionine (D <sub>8</sub> , 98%; <sup>15</sup> N, 98%)	0.25 g
CDNLM-6798	L-Methionine ( <sup>13</sup> C <sub>5</sub> , 97-99%; D <sub>8</sub> , 97-99%; <sup>15</sup> N, 97-99%)	Please inquire
CLM-1036	L-Ornithine·HCl (1,2- <sup>13</sup> C <sub>2</sub> , 99%)	Please inquire
CLM-4724-H	L-Ornithine·HCl ( <sup>13</sup> C <sub>5</sub> , 99%)	0.1 g
DLM-4261	L-Ornithine·HCl (5,5-D <sub>2</sub> , 98%)	0.25 g
DLM-6046	L-Ornithine·HCl (4,4,5,5-D <sub>4</sub> , 95%)	Please inquire
DLM-2969	L-Ornithine·HCl (3,3,4,4,5,5-D <sub>6</sub> , 98%)	0.1 g, 0.25 g
DLM-6669	L-Ornithine·HCl (D <sub>7</sub> , 98%)	0.25 g
NLM-2212	L-Ornithine·HCl (α- <sup>15</sup> N, 98%)	Please inquire
NLM-2174	L-Ornithine·HCl (5- <sup>15</sup> N, 98%)	Please inquire
NLM-3610	L-Ornithine·HCl ( <sup>15</sup> N <sub>2</sub> , 98%)	0.25 g
CDLM-3873	L-Ornithine·HCl (5- <sup>13</sup> C, 99%; 4,4,5,5-D <sub>4</sub> , 95%)	Please inquire
CNLM-7578-H	L-Ornithine·HCl ( <sup>13</sup> C <sub>5</sub> , 99%; <sup>15</sup> N <sub>2</sub> , 99%)	Please inquire
CLM-762	L-Phenylalanine (1- <sup>13</sup> C, 99%)	1 g
CLM-1631	L-Phenylalanine (2- <sup>13</sup> C, 99%) CP 97%	0.05 g, 0.25 g
CLM-1053	L-Phenylalanine (3- <sup>13</sup> C, 99%)	0.1 g, 0.25 g
CLM-1055	L-Phenylalanine (ring- <sup>13</sup> C <sub>6</sub> , 99%)	0.25 g
CLM-2250-H	L-Phenylalanine ( <sup>13</sup> C <sub>9</sub> , 99%)	0.25 g, 0.5 g, 1 g
DLM-2984	L-Phenylalanine (2-D, 95%)	0.5 g
DLM-2985	L-Phenylalanine (3,3-D <sub>2</sub> , 98%)	0.1 g, 0.5 g, 1 g
DLM-1258	L-Phenylalanine (ring-D <sub>5</sub> , 98%)	1 g, 5 g
DLM-372	L-Phenylalanine (D <sub>8</sub> , 98%)	1 g
NLM-108	L-Phenylalanine ( <sup>15</sup> N, 98%)	0.5 g, 1 g
CNLM-7611	L-Phenylalanine (2,3- <sup>13</sup> C <sub>2</sub> , 99%; <sup>15</sup> N, 98%)	Please inquire
CNLM-575-H	L-Phenylalanine ( <sup>13</sup> C <sub>9</sub> , 99%; <sup>15</sup> N, 99%)	0.1 g, 0.25 g, 0.5 g, 1 g

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## Amino Acids (continued)

Catalog No.	Description	Unit Size
DNLM-7180	L-Phenylalanine (D <sub>8</sub> , 98%; <sup>15</sup> N, 98%)	0.25 g, 0.5 g
CDNLM-6811	L-Phenylalanine ( <sup>13</sup> C <sub>9</sub> , 97-99%; D <sub>8</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.25 g
CLM-510	L-Proline (1- <sup>13</sup> C, 99%)	0.25 g
CLM-2260-H	L-Proline ( <sup>13</sup> C <sub>5</sub> , 99%)	0.1 g, 0.25 g, 0.5 g
DLM-487	L-Proline (D <sub>7</sub> , 97-98%)	0.1 g, 0.25 g
NLM-835	L-Proline ( <sup>15</sup> N, 98%)	0.25 g, 0.5 g
CNLM-7822	L-Proline (1- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	Please inquire
CNLM-436-H	L-Proline ( <sup>13</sup> C <sub>5</sub> , 99%; <sup>15</sup> N, 99%)	0.1 g, 0.25 g, 0.5 g
DNLM-7562	L-Proline (D <sub>7</sub> , 98%; <sup>15</sup> N, 98%)	0.25 g
CDNLM-6812	L-Proline ( <sup>13</sup> C <sub>5</sub> , 97-99%; D <sub>7</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.25 g
CLM-1573	L-Serine (1- <sup>13</sup> C, 99%)	0.25 g
CLM-2013	L-Serine (2- <sup>13</sup> C, 99%)	0.1 g
CLM-1572	L-Serine (3- <sup>13</sup> C, 99%)	0.1 g, 0.25 g
CLM-1574-H	L-Serine ( <sup>13</sup> C <sub>3</sub> , 99%)	0.1 g, 0.25 g, 0.5 g
DLM-161	L-Serine (3,3-D <sub>2</sub> , 98%)	Please inquire
DLM-582	L-Serine (2,3,3-D <sub>3</sub> , 98%)	0.1 g, 0.5 g
NLM-2036	L-Serine ( <sup>15</sup> N, 98%)	0.5 g, 1 g
CNLM-7814	L-Serine (1- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	0.25 g
CNLM-474-H	L-Serine ( <sup>13</sup> C <sub>3</sub> , 99%; <sup>15</sup> N, 99%)	0.1 g, 0.25 g, 0.5 g
DNLM-6863	L-Serine (2,3,3-D <sub>3</sub> , 98%; <sup>15</sup> N, 98%)	0.25 g
CDNLM-6813	L-Serine ( <sup>13</sup> C <sub>3</sub> , 97-99%; D <sub>3</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.25 g
CLM-447	L-Threonine (1- <sup>13</sup> C, 99%)	0.5 g
CLM-2261	L-Threonine ( <sup>13</sup> C <sub>4</sub> , 97-99%)	0.1 g, 0.25 g, 0.5 g
DLM-1693	L-Threonine (D <sub>5</sub> , 98%)	Please inquire
NLM-742	L-Threonine ( <sup>15</sup> N, 98%)	0.25 g, 0.5 g
CDLM-9307	L-Threonine (4- <sup>13</sup> C, 97%; 2,3-D <sub>2</sub> , 98%)	0.1 g, 0.5 g
CNLM-587	L-Threonine ( <sup>13</sup> C <sub>4</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.1 g, 0.25 g, 0.5 g
DNLM-7367	L-Threonine (D <sub>5</sub> , 97%; <sup>15</sup> N, 98%)	0.25 g, 0.5 g
CDNLM-6814	L-Threonine ( <sup>13</sup> C <sub>4</sub> , 97-99%; D <sub>5</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.25 g
CLM-778	L-Tryptophan (1- <sup>13</sup> C, 99%)	0.25 g
CLM-1543	L-Tryptophan (indole-2- <sup>13</sup> C, 98%)	0.25 g
CLM-716	L-Tryptophan (indole-3- <sup>13</sup> C, 95-99%)	0.25 g
CLM-717	L-Tryptophan (indole-4- <sup>13</sup> C, 99%) CP 95%	Please inquire
CLM-4290-H	L-Tryptophan ( <sup>13</sup> C <sub>11</sub> , 99%)	0.1 g
DLM-1092	L-Tryptophan (indole-D <sub>5</sub> , 98%)	0.5 g
DLM-6903	L-Tryptophan (D <sub>8</sub> , 97-98%)	0.25 g
NLM-1695	L-Tryptophan (α- <sup>15</sup> N, 95-99%)	0.1 g
NLM-1208	L-Tryptophan (indole- <sup>15</sup> N, 98%)	0.25 g, 0.5 g
NLM-800	L-Tryptophan ( <sup>15</sup> N <sub>2</sub> , 98%)	0.25 g, 0.5 g
CNLM-2475-H	L-Tryptophan ( <sup>13</sup> C <sub>11</sub> , 99%; <sup>15</sup> N <sub>2</sub> , 99%)	0.1 g
DNLM-6904	L-Tryptophan (D <sub>8</sub> , 98%; <sup>15</sup> N <sub>2</sub> , 98%)	0.25 g
CDNLM-6816	L-Tryptophan ( <sup>13</sup> C <sub>11</sub> , 97-99%; D <sub>8</sub> , 97-99%; <sup>15</sup> N <sub>2</sub> , 97-99%)	0.25 g
CLM-776	L-Tyrosine (1- <sup>13</sup> C, 99%)	1 g
CLM-437	L-Tyrosine (2- <sup>13</sup> C, 99%)	0.1 g, 0.25 g
CLM-3378	L-Tyrosine (3- <sup>13</sup> C, 99%)	0.1 g, 0.25 g
CLM-622	L-Tyrosine (phenol-4- <sup>13</sup> C, 95-99%)	0.25 g
CLM-623	L-Tyrosine (phenol-3,5- <sup>13</sup> C <sub>2</sub> , 95-99%)	0.25 g
CLM-1542	L-Tyrosine (ring- <sup>13</sup> C <sub>6</sub> , 99%)	0.25 g
CLM-2263-H	L-Tyrosine ( <sup>13</sup> C <sub>9</sub> , 99%)	0.1 g, 0.25 g, 0.5 g
DLM-2317	L-Tyrosine (3,3-D <sub>2</sub> , 98%)	0.5 g, 1 g
DLM-449	L-Tyrosine (ring-3,5-D <sub>2</sub> , 98%)	1 g, 5 g
DLM-2917	L-Tyrosine (ring-2,6-D <sub>2</sub> , 2-D, 98%)	Please inquire

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Catalog No.	Description	Unit Size
DLM-451	L-Tyrosine (ring-D <sub>4</sub> , 98%)	0.5 g, 1 g
DLM-589	L-Tyrosine (D <sub>7</sub> , 98%)	Please inquire
NLM-590	L-Tyrosine ( <sup>15</sup> N, 98%)	0.5 g
CDLM-2369	L-Tyrosine (ring- <sup>13</sup> C <sub>6</sub> , 99%; 3,3-D <sub>2</sub> , 30%)	0.1 g
CNLM-7809	L-Tyrosine (1- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	0.25 g
CNLM-7610	L-Tyrosine (2,3- <sup>13</sup> C <sub>2</sub> , 99%; <sup>15</sup> N, 98%)	Please inquire
CNLM-439-H	L-Tyrosine ( <sup>13</sup> C <sub>9</sub> , 99%; <sup>15</sup> N, 99%)	0.1 g, 0.25 g, 0.5 g
DNLM-7373	L-Tyrosine (D <sub>7</sub> , 98%; <sup>15</sup> N, 98%)	0.25 g
NOLM-10743	L-Tyrosine ( <sup>15</sup> N, 98%; carboxyl- <sup>17</sup> O <sub>2</sub> , 60%)	Please inquire
CDNLM-6815	L-Tyrosine ( <sup>13</sup> C <sub>9</sub> , 97-99%; D <sub>7</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.25 g
CLM-470	L-Valine (1- <sup>13</sup> C, 99%)	1 g
CLM-3050	L-Valine (2- <sup>13</sup> C, 99%)	0.25 g
CLM-9217	L-Valine (dimethyl- <sup>13</sup> C <sub>2</sub> , 99%)	0.25 g, 1 g
CLM-2249-H	L-Valine ( <sup>13</sup> C <sub>5</sub> , 99%)	0.25 g, 1 g, 5 g
DLM-7732	L-Valine (3-D, 98%)	1 g
DLM-4364	L-Valine (2,3-D <sub>2</sub> , 98%)	0.1 g, 0.25 g
DLM-488	L-Valine (D <sub>8</sub> , 98%)	0.25 g, 0.5 g
NLM-316	L-Valine ( <sup>15</sup> N, 98%)	0.5 g, 1 g
CNLM-3466	L-Valine (1- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	0.25 g
CNLM-8678	L-Valine (2- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	Please inquire
CNLM-442-H	L-Valine ( <sup>13</sup> C <sub>5</sub> , 99%; <sup>15</sup> N, 99%)	0.25 g, 0.5 g, 1 g
DNLM-4643	L-Valine (D <sub>8</sub> , 96%; <sup>15</sup> N, 96%)	0.25 g, 0.5 g
NOLM-10742	L-Valine ( <sup>15</sup> N, 98%; carboxyl- <sup>17</sup> O <sub>2</sub> , 60%)	Please inquire
CDNLM-4281	L-Valine ( <sup>13</sup> C <sub>5</sub> , 95-97%; 2,3-D <sub>2</sub> , 97%; <sup>15</sup> N, 96-99%)	0.1 g, 0.25 g
CDNLM-6817	L-Valine ( <sup>13</sup> C <sub>5</sub> , 97-99%; D <sub>8</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.25 g

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## Protected Amino Acids

CIL offers a comprehensive list of labeled protected amino acids for use as starting materials for peptide synthesis. The large number of available labeling schemes maximizes flexibility involved in designing experiments to probe structure and dynamics alone or in the presence of biological macromolecules. Pricing and availability can be found on [isotope.com](http://isotope.com).

Catalog No.	Description	Unit Size
CLM-818	L-Alanine- <i>N</i> -FMOC (1- <sup>13</sup> C, 99%)	1 g
CLM-3638	L-Alanine- <i>N</i> -FMOC (2- <sup>13</sup> C, 99%)	0.25 g
CLM-1142	L-Alanine- <i>N</i> -FMOC (3- <sup>13</sup> C, 99%)	1 g
CLM-7785	L-Alanine- <i>N</i> -FMOC ( <sup>13</sup> C <sub>3</sub> , 97-99%)	0.25 g
DLM-7316	L-Alanine- <i>N</i> -FMOC (3,3,3-D <sub>3</sub> , 98%)	1 g
DLM-8168	L-Alanine- <i>N</i> -FMOC (2,3,3,3-D <sub>4</sub> , 98%)	0.5 g
NLM-614	L-Alanine- <i>N</i> -FMOC ( <sup>15</sup> N, 98%)	1 g
CNLM-4355-H	L-Alanine- <i>N</i> -FMOC ( <sup>13</sup> C <sub>3</sub> , 99%; <sup>15</sup> N, 99%)	0.1 g, 0.25 g
CDNLM-7852	L-Alanine- <i>N</i> -FMOC ( <sup>13</sup> C <sub>3</sub> , 97-99%; D <sub>4</sub> , 97-99%; <sup>15</sup> N, 97-99%)	Please inquire
CLM-2150	L-Alanine- <i>N</i> - <i>t</i> -BOC (1- <sup>13</sup> C, 99%)	1 g
CLM-2011	L-Alanine- <i>N</i> - <i>t</i> -BOC (2- <sup>13</sup> C, 98-99%)	0.25 g
CLM-2151	L-Alanine- <i>N</i> - <i>t</i> -BOC (3- <sup>13</sup> C, 99%)	0.5 g, 1 g
CLM-3589	L-Alanine- <i>N</i> - <i>t</i> -BOC ( <sup>13</sup> C <sub>3</sub> , 97-99%)	0.25 g
DLM-649	L-Alanine- <i>N</i> - <i>t</i> -BOC (2-D, 98%)	Please inquire
DLM-2793	L-Alanine- <i>N</i> - <i>t</i> -BOC (3,3,3-D <sub>3</sub> , 99%)	1 g
NLM-1903	L-Alanine- <i>N</i> - <i>t</i> -BOC ( <sup>15</sup> N, 98%)	0.25 g, 0.5 g, 1 g
CNLM-6014	L-Alanine- <i>N</i> - <i>t</i> -BOC (2- <sup>13</sup> C, 99%; <sup>15</sup> N, 96-99%)	Please inquire
CNLM-2394	L-Alanine- <i>N</i> - <i>t</i> -BOC ( <sup>13</sup> C <sub>3</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.05 g, 0.1 g
CLM-8475-H	L-Arginine- <i>N</i> -FMOC, PBF-OH ( <sup>13</sup> C <sub>6</sub> , 99%) contains solvent	1 g
NLM-8841	L-Arginine- <i>N</i> -FMOC, PBF-OH ( <sup>15</sup> N <sub>4</sub> , 98%) contains solvent	Please inquire
CNLM-8474-H	L-Arginine- <i>N</i> -FMOC, PBF-OH ( <sup>13</sup> C <sub>6</sub> , 99%; <sup>15</sup> N <sub>4</sub> , 99%) contains solvent	0.1 g, 0.25 g, 0.5 g, 1 g
NLM-1264	L-Arginine- <i>N</i> -FMOC, PMC ( <sup>15</sup> N <sub>4</sub> , 98%) CP 94%	0.1 g
CNLM-4354	L-Asparagine- <i>N</i> -FMOC ( <sup>13</sup> C <sub>4</sub> , 97-99%; <sup>15</sup> N <sub>2</sub> , 97-99%)	Please inquire
CNLM-6193-H	L-Asparagine- <i>N</i> -FMOC, <i>N</i> -β-trityl ( <sup>13</sup> C <sub>4</sub> , 99%; <sup>15</sup> N <sub>2</sub> , 99%)	0.1 g
NLM-4204	L-Asparagine- <i>N</i> -FMOC, <i>N</i> -β-trityl ( <sup>15</sup> N <sub>2</sub> , 98%)	0.1 g
CNLM-4788	L-Aspartic acid- <i>N</i> -FMOC ( <sup>13</sup> C <sub>4</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.05 g
NLM-647	L-Aspartic acid- <i>N</i> -FMOC, β- <i>O</i> - <i>t</i> -butyl ester ( <sup>15</sup> N, 98%)	0.1 g, 0.5 g, 1 g
NLM-3493	L-Aspartic acid- <i>N</i> - <i>t</i> -BOC ( <sup>15</sup> N, 98%)	1 g
NLM-1908	L-Aspartic acid- <i>N</i> - <i>t</i> -BOC, β-Bz ester ( <sup>15</sup> N, 98%)	0.25 g
CNLM-2392	L-Aspartic acid- <i>N</i> - <i>t</i> -BOC, β-Bz ester ( <sup>13</sup> C <sub>4</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.05 g
DLM-4721	L-Cysteine- <i>N</i> -FMOC, <i>S</i> -trityl (3,3-D <sub>2</sub> , 98%)	0.1 g, 0.25 g, 0.5 g
CNLM-4722-H	L-Cysteine- <i>N</i> -FMOC, <i>S</i> -trityl ( <sup>13</sup> C <sub>3</sub> , 99%; <sup>15</sup> N, 99%)	0.1 g, 0.25 g
CLM-1901	L-Cysteine- <i>N</i> - <i>t</i> -BOC, <i>S</i> -benzyl (3- <sup>13</sup> C, 99%)	0.25 g
NLM-3874	L-Cysteine- <i>N</i> - <i>t</i> -BOC, <i>S</i> - <i>P</i> -mebz ( <sup>15</sup> N, 98%)	0.25 g
NLM-8960	L-Glutamic acid- <i>N</i> -FMOC, γ- <i>t</i> -butyl ester ( <sup>15</sup> N, 98%)	0.1 g
CNLM-4753-H	L-Glutamic acid- <i>N</i> -FMOC, γ- <i>t</i> -butyl ester ( <sup>13</sup> C <sub>5</sub> , 99%; <sup>15</sup> N, 99%) CP 96%	0.1 g
CLM-2008	L-Glutamic acid- <i>N</i> - <i>t</i> -BOC, γ-benzyl ester (1,2- <sup>13</sup> C <sub>2</sub> , 99%)	0.1 g
NLM-1907	L-Glutamic acid- <i>N</i> - <i>t</i> -BOC, γ-benzyl ester ( <sup>15</sup> N, 98%)	Please inquire
CLM-1902	L-Glutamine- <i>N</i> - <i>t</i> -BOC (1,2- <sup>13</sup> C <sub>2</sub> , 99%)	0.1 g
NLM-3419	L-Glutamine- <i>N</i> - <i>t</i> -BOC (α- <sup>15</sup> N, 98%)	0.5 g
CLM-3639	Glycine- <i>N</i> -FMOC (1- <sup>13</sup> C, 99%)	1 g
CLM-2387	Glycine- <i>N</i> -FMOC (2- <sup>13</sup> C, 99%)	1 g
CLM-7547	Glycine- <i>N</i> -FMOC ( <sup>13</sup> C <sub>2</sub> , 97-99%)	1 g
DLM-7339	Glycine- <i>N</i> -FMOC (2,2-D <sub>2</sub> , 98%)	1 g
NLM-694	Glycine- <i>N</i> -FMOC ( <sup>15</sup> N, 98%)	1 g
CNLM-7697	Glycine- <i>N</i> -FMOC (1- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	0.25 g
CNLM-7698	Glycine- <i>N</i> -FMOC (2- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	0.1 g
CNLM-4357-H	Glycine- <i>N</i> -FMOC ( <sup>13</sup> C <sub>2</sub> , 99%; <sup>15</sup> N, 99%)	0.1 g, 0.25 g
CDNLM-7853	Glycine- <i>N</i> -FMOC ( <sup>13</sup> C <sub>2</sub> , 97-99%; 2,2-D <sub>2</sub> , 97-99%; <sup>15</sup> N, 97-99%)	Please inquire

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Catalog No.	Description	Unit Size
CLM-2152	Glycine- <i>N</i> - <i>t</i> -BOC (1- <sup>13</sup> C, 99%)	1 g
CLM-1900	Glycine- <i>N</i> - <i>t</i> -BOC (2- <sup>13</sup> C, 99%)	1 g
DLM-2153	Glycine- <i>N</i> - <i>t</i> -BOC (2,2-D <sub>2</sub> , 98%)	1 g
NLM-2109	Glycine- <i>N</i> - <i>t</i> -BOC ( <sup>15</sup> N, 98%)	1 g
CNLM-9686	Glycine- <i>N</i> - <i>t</i> -BOC (2- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%)	Please inquire
CNLM-2412	Glycine- <i>N</i> - <i>t</i> -BOC ( <sup>13</sup> C <sub>2</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.1 g
NLM-8010	L-Histidine- <i>N</i> -FMOC, <i>N</i> -Im-trityl ( <sup>15</sup> N <sub>3</sub> , 98%)	0.1 g
CLM-8043	L-Isoleucine- <i>N</i> -FMOC (1- <sup>13</sup> C, 99%)	0.25 g
CLM-7794	L-Isoleucine- <i>N</i> -FMOC ( <sup>13</sup> C <sub>6</sub> , 97-99%)	Please inquire
NLM-391	L-Isoleucine- <i>N</i> -FMOC ( <sup>15</sup> N, 98%)	0.25 g
NLM-2167	L-Isoleucine- <i>N</i> - <i>t</i> -BOC ( <sup>15</sup> N, 98%)	0.25 g
CLM-3691	L-Leucine- <i>N</i> -FMOC (1- <sup>13</sup> C, 99%)	1 g
CLM-7546	L-Leucine- <i>N</i> -FMOC (1,2- <sup>13</sup> C <sub>2</sub> , 99%)	0.1 g
CLM-3683	L-Leucine- <i>N</i> -FMOC ( <sup>13</sup> C <sub>6</sub> , 97-99%)	0.1 g
DLM-7202	L-Leucine- <i>N</i> -FMOC (5,5,5-D <sub>3</sub> , 98%)	1 g
DLM-7575	L-Leucine- <i>N</i> -FMOC (D <sub>10</sub> , 98%)	0.25 g
NLM-2397	L-Leucine- <i>N</i> -FMOC ( <sup>15</sup> N, 98%)	1 g
CNLM-4345-H	L-Leucine- <i>N</i> -FMOC ( <sup>13</sup> C <sub>6</sub> , 99%; <sup>15</sup> N, 99%)	0.1 g, 0.25 g
CDNLM-7854	L-Leucine- <i>N</i> -FMOC ( <sup>13</sup> C <sub>6</sub> , 97-99%; D <sub>10</sub> , 97-99%; <sup>15</sup> N, 97-99%)	Please inquire
CLM-2155	L-Leucine- <i>N</i> - <i>t</i> -BOC·H <sub>2</sub> O (1- <sup>13</sup> C, 99%)	1 g
CLM-2010	L-Leucine- <i>N</i> - <i>t</i> -BOC·H <sub>2</sub> O (2- <sup>13</sup> C, 99%)	0.25 g
DLM-2736	L-Leucine- <i>N</i> - <i>t</i> -BOC·H <sub>2</sub> O (5,5,5-D <sub>3</sub> , 98%)	1 g
DLM-3650	L-Leucine- <i>N</i> - <i>t</i> -BOC·H <sub>2</sub> O (D <sub>10</sub> , 98%)	0.5 g
NLM-1904	L-Leucine- <i>N</i> - <i>t</i> -BOC·H <sub>2</sub> O ( <sup>15</sup> N, 98%)	1 g
CNLM-2396	L-Leucine- <i>N</i> - <i>t</i> -BOC·H <sub>2</sub> O ( <sup>13</sup> C <sub>6</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.05 g
CLM-6194	L-Lysine- $\alpha$ - <i>N</i> -FMOC, $\epsilon$ - <i>N</i> - <i>t</i> -BOC (1- <sup>13</sup> C, 99%)	0.1 g
CLM-7865-H	L-Lysine- $\alpha$ - <i>N</i> -FMOC, $\epsilon$ - <i>N</i> - <i>t</i> -BOC ( <sup>13</sup> C <sub>6</sub> , 99%)	Please inquire
NLM-4631	L-Lysine- $\alpha$ - <i>N</i> -FMOC, $\epsilon$ - <i>N</i> - <i>t</i> -BOC ( <sup>15</sup> N <sub>2</sub> , 96-98%)	0.1 g
CNLM-4754-H	L-Lysine- $\alpha$ - <i>N</i> -FMOC, $\epsilon$ - <i>N</i> - <i>t</i> -BOC ( <sup>13</sup> C <sub>6</sub> , 99%; <sup>15</sup> N <sub>2</sub> , 99%)	0.1 g, 0.25 g, 0.5 g, 1 g
CLM-8166	L-Methionine- <i>N</i> -FMOC (1- <sup>13</sup> C, 99%)	Please inquire
CLM-1141	L-Methionine- <i>N</i> -FMOC (methyl- <sup>13</sup> C, 99%)	Please inquire
NLM-4632	L-Methionine- <i>N</i> -FMOC ( <sup>15</sup> N, 98%) CP 95%	Please inquire
CNLM-4358-H	L-Methionine- <i>N</i> -FMOC ( <sup>13</sup> C <sub>5</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.1 g
CLM-2156	L-Methionine- <i>N</i> - <i>t</i> -BOC (methyl- <sup>13</sup> C, 98%)	Please inquire
CLM-4824	L-Phenylalanine- <i>N</i> -FMOC (1- <sup>13</sup> C, 99%)	0.5 g
CLM-3684	L-Phenylalanine- <i>N</i> -FMOC (ring- <sup>13</sup> C <sub>6</sub> , 99%)	0.1 g
DLM-7786	L-Phenylalanine- <i>N</i> -FMOC (ring-D <sub>5</sub> , 98%)	0.25 g
DLM-8752	L-Phenylalanine- <i>N</i> -FMOC (D <sub>8</sub> , 98%)	Please inquire
NLM-1265	L-Phenylalanine- <i>N</i> -FMOC ( <sup>15</sup> N, 98%)	1 g
CNLM-4362-H	L-Phenylalanine- <i>N</i> -FMOC ( <sup>13</sup> C <sub>9</sub> , 99%; <sup>15</sup> N, 99%)	0.1 g, 0.25 g
CLM-2170	L-Phenylalanine- <i>N</i> - <i>t</i> -BOC (1- <sup>13</sup> C, 99%)	0.5 g
CLM-2009	L-Phenylalanine- <i>N</i> - <i>t</i> -BOC (2- <sup>13</sup> C, 99%)	0.25 g
CLM-2061	L-Phenylalanine- <i>N</i> - <i>t</i> -BOC (ring- <sup>13</sup> C <sub>6</sub> , 99%)	0.1 g
CLM-7859	L-Phenylalanine- <i>N</i> - <i>t</i> -BOC ( <sup>13</sup> C <sub>9</sub> , 97-99%)	0.05 g
DLM-2157	L-Phenylalanine- <i>N</i> - <i>t</i> -BOC (ring-D <sub>5</sub> , 98%)	1 g
NLM-1905	L-Phenylalanine- <i>N</i> - <i>t</i> -BOC ( <sup>15</sup> N, 98%)	1 g
CNLM-2393	L-Phenylalanine- <i>N</i> - <i>t</i> -BOC ( <sup>13</sup> C <sub>9</sub> , 97-99%; <sup>15</sup> N, 97-99%)	0.05 g
CLM-8044	L-Proline- <i>N</i> -FMOC (1- <sup>13</sup> C, 99%)	0.25 g
NLM-3906	L-Proline- <i>N</i> -FMOC ( <sup>15</sup> N, 98%)	0.25 g
NLM-2329	L-Proline- <i>N</i> - <i>t</i> -BOC ( <sup>15</sup> N, 96%)	0.25 g
CNLM-4347-H	L-Proline- <i>N</i> -FMOC ( <sup>13</sup> C <sub>5</sub> , 99%; <sup>15</sup> N, 97-99%)	0.1 g, 0.25 g

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## Protected Amino Acids (continued)

Cata log No.	Description	Unit Size
CNLM-8403-H	L-Serine- <i>N</i> -FMOC ( $^{13}\text{C}_3$ , 99%; $^{15}\text{N}$ , 99%)	1 g
CLM-8167	L-Serine- <i>N</i> -FMOC, <i>O</i> - <i>t</i> -butyl ether ( $1\text{-}^{13}\text{C}$ , 99%)	0.25 g
NLM-4630	L-Serine- <i>N</i> -FMOC, <i>O</i> - <i>t</i> -butyl ether ( $^{15}\text{N}$ , 98%)	0.25 g
CNLM-4755-H	L-Serine- <i>N</i> -FMOC, <i>O</i> - <i>t</i> -butyl ether ( $^{13}\text{C}_3$ , 99%; $^{15}\text{N}$ , 99%)	0.1 g
CLM-2007	L-Serine- <i>N</i> - <i>t</i> -BOC, <i>O</i> -Bz ether ( $2\text{-}^{13}\text{C}$ , 99%)	Please inquire
CLM-756	L-Serine- <i>N</i> - <i>t</i> -BOC, <i>O</i> -Bz ether ( $3\text{-}^{13}\text{C}$ , 99%)	0.1 g
NLM-2025	L-Serine- <i>N</i> - <i>t</i> -BOC, <i>O</i> -Bz ether ( $^{15}\text{N}$ , 98%)	0.1 g
NLM-8170	L-Threonine- <i>N</i> -FMOC, <i>O</i> - <i>t</i> -butyl ether ( $^{15}\text{N}$ , 98%)	0.1 g
CNLM-7615-H	L-Threonine- <i>N</i> -FMOC, <i>O</i> - <i>t</i> -butyl ether ( $^{13}\text{C}_4$ , 99%; $^{15}\text{N}$ , 99%)	0.1 g
NLM-3681	L-Threonine- <i>N</i> - <i>t</i> -BOC, <i>O</i> -benzyl ether ( $^{15}\text{N}$ , 98%)	Please inquire
DLM-6113	L-Tryptophan- <i>N</i> -FMOC (indole- $\text{D}_5$ , 98%)	0.25 g
NLM-3423	L-Tryptophan- <i>N</i> -FMOC ( $\alpha\text{-}^{15}\text{N}$ , 98%)	Please inquire
CNLM-6077	L-Tryptophan- <i>N</i> -FMOC ( $^{13}\text{C}_{11}$ , 97-99%; $^{15}\text{N}_2$ , 97-99%)	0.1 g
CLM-2194	L-Tryptophan- <i>N</i> - <i>t</i> -BOC ( $1\text{-}^{13}\text{C}$ , 99%)	Please inquire
CNLM-4349-H	L-Tyrosine- <i>N</i> -FMOC, <i>O</i> - <i>t</i> -butyl ether ( $^{13}\text{C}_9$ , 99%; $^{15}\text{N}$ , 99%)	0.1 g, 0.25 g
NLM-8169	L-Tyrosine- <i>N</i> -FMOC, <i>O</i> - <i>t</i> -butyl ether ( $^{15}\text{N}$ , 98%)	0.1 g
DLM-2303	L-Tyrosine- <i>N</i> - <i>t</i> -BOC, <i>O</i> -Bz ether (ring- $\text{D}_4$ , 98%)	0.25 g
NLM-1906	L-Tyrosine- <i>N</i> - <i>t</i> -BOC, <i>O</i> -Bz ether ( $^{15}\text{N}$ , 98%)	0.25 g
CLM-3640	L-Valine- <i>N</i> -FMOC ( $1\text{-}^{13}\text{C}$ , 99%)	1 g
CLM-7793	L-Valine- <i>N</i> -FMOC ( $^{13}\text{C}_5$ , 97-99%)	0.1 g
DLM-7784	L-Valine- <i>N</i> -FMOC ( $\text{D}_8$ , 98%)	0.5 g
NLM-4243	L-Valine- <i>N</i> -FMOC ( $^{15}\text{N}$ , 98%)	1 g
CNLM-4348-H	L-Valine- <i>N</i> -FMOC ( $^{13}\text{C}_5$ , 99%; $^{15}\text{N}$ , 99%)	0.1 g, 0.25 g
CLM-2158	L-Valine- <i>N</i> - <i>t</i> -BOC ( $1\text{-}^{13}\text{C}$ , 99%)	Please inquire
DLM-3651	L-Valine- <i>N</i> - <i>t</i> -BOC ( $\text{D}_8$ , 98%)	0.5 g
NLM-2060	L-Valine- <i>N</i> - <i>t</i> -BOC ( $^{15}\text{N}$ , 98%)	0.5 g, 1 g
CNLM-2395	L-Valine- <i>N</i> - <i>t</i> -BOC ( $^{13}\text{C}_5$ , 97-99%; $^{15}\text{N}$ , 97-99%)	0.05 g

“Over the past two decades, companies providing specifically labeled isotopes and precursors have changed our ways and understanding of biomolecular NMR sample preparations. CIL, being the widely popular company, certainly plays a crucial role in this development. I enjoy working with CIL because of the value, quality of products and superb service. I have been ordering from them for the past 15+ years: from Munich as a PhD student, from San Francisco as post-doc, and from Hyderabad as a PI. To tell you the truth, I do not want to even imagine an NMR world without isotopes and CIL.”

Mandar V. Deshmukh, PhD  
 Scientist, Structural Biology (NMR)  
 Centre for Cellular and Molecular Biology

## Sparse Labeling Reagents

The presence of one bond  $^{13}\text{C}$ - $^{13}\text{C}$  coupling in proteins may present difficulties in obtaining structural information in both solid-state and solution NMR. It is easier to overcome these difficulties by simply analyzing sparsely  $^{13}\text{C}$ -labeled protein than resorting to using specialized pulse sequences. Sparsely labeled protein is made by expressing protein in *E. coli* grown using selectively  $^{13}\text{C}$ -labeled carbon sources. The use of 2- $^{13}\text{C}$  and 1,3- $^{13}\text{C}_2$  glycerol, as originally described by LeMaster,<sup>1</sup> remains to be the most popular sparse labeling method in current use. Proteins expressed using 1- $^{13}\text{C}$  glucose and 2- $^{13}\text{C}$  glucose as sole carbon sources, however, have been used to determine intermolecular distances in supramolecular complexes using  $^{13}\text{C}$ - $^{13}\text{C}$  solid state NMR.<sup>2</sup> CIL offers the following selectively  $^{13}\text{C}$ -labeled *E. coli* carbon sources.

### Sparse Labeling Reagents

Catalog No.	Description
CLM-420	D-Glucose (1- $^{13}\text{C}$ , 99%)
CLM-746	D-Glucose (2- $^{13}\text{C}$ , 98-99%)
CLM-1396-25-1	D-Glucose ( $^{13}\text{C}_6$ , 24-25%)
CLM-1397	Glycerol (2- $^{13}\text{C}$ , 99%)
CLM-1857	Glycerol (1,3- $^{13}\text{C}_2$ , 99%)
CLM-1580	Sodium pyruvate (2- $^{13}\text{C}$ , 99%)
CLM-1575	Sodium pyruvate (3- $^{13}\text{C}$ , 99%)

#### References

- Wylie, B.J.; Schwieters, C.D.; Oldfield, E.; et al. **2009**. Protein structure refinement using  $^{13}\text{C}\alpha$  chemical shift tensors. *J Am Chem Soc*, 131(3), 985-992.
- Loquet, A.; Giller, K.; Becker, S.; et al. **2010**. Supramolecular interactions probed by  $^{13}\text{C}$ - $^{13}\text{C}$  solid-state NMR spectroscopy. *J Am Chem Soc*, 132, 15164-15166.

## Mixed Pyruvate Labeling

A backbone resonance assignment strategy was recently published<sup>1</sup> which describes the use of an equimolar mix of 2- $^{13}\text{C}$  and 3- $^{13}\text{C}$  sodium pyruvate as the sole carbon source for the expression of recombinant  $^{13}\text{C}$  sparsely labeled protein in *E. coli* cells. The achieved sparse  $^{13}\text{C}$  labeling suppresses one-bond ( $1J_{\alpha\beta}$ ) coupling, which provides enhanced resolution for the  $\text{C}\alpha$  resonance and results in distinct peak shapes for each amino acid in 1D traces along the  $\text{C}\alpha$  dimension of 3D HNCA spectra. This method allows the near total assignment of backbone resonances of large proteins using a single HNCA experiment, reducing the required spectrometer time compared to current resonance assignment procedures.

CIL is pleased to assist researchers using this technique by supplying prepackaged amounts of 2- $^{13}\text{C}$  and 3- $^{13}\text{C}$  sodium pyruvate in a bundled packaged, referred to as "Hari's Mix."

### Pyruvate $^{13}\text{C}$ Labeling Mix

Catalog No.	Description
CLM-10978-KIT <b>NEW!</b>	1 vial containing 2- $^{13}\text{C}$ sodium pyruvate (1.5 g) (CLM-1580) 1 vial containing 3- $^{13}\text{C}$ sodium pyruvate (1.5 g) (CLM-1575)

#### References

- Robson, S.A.; Takeuchi, K.; Boeszoermenyi, A.; et al. **2018**. Mixed pyruvate labeling enables backbone resonance assignment of large proteins using a single experiment. *Nat Commun*, 9(1), 356.

### Recommended Preparation of Growth Media with Deuterated Mixed Pyruvates\*

- Step 1.** In a suitable container, dissolve contents of both vials in 1 kg of  $\text{D}_2\text{O}$  (99.9%) (DLM-4).
- Step 2.** Adjust pH to ~13 by adding 2.5 mM NaOD (prepared using 40% NaOD in  $\text{D}_2\text{O}$  – DLM-45), while stirring. Wait 30 minutes, stirring occasionally.
- Step 3.** Add 4.26 g  $\text{Na}_2\text{HPO}_4$ , 3.6 g  $\text{NaH}_2\text{PO}_4$ , and 3 g  $\text{KH}_2\text{PO}_4$  to reach pH 7.
- Step 4.** Add remaining compounds to medium. These ingredients will generally include ammonium chloride, sodium bicarbonate, magnesium sulfate, calcium chloride, and antibiotics.
- Step 5.** Sterile filter the medium prior to use.

\*This medium is not compatible with rich media additives, such as Celtone or BioExpress 1000.

## Deuterated Detergents and Phospholipids for Membrane Proteins

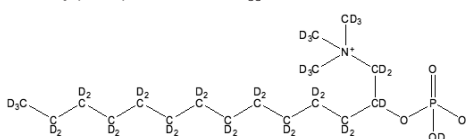
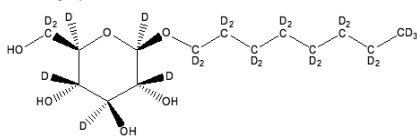
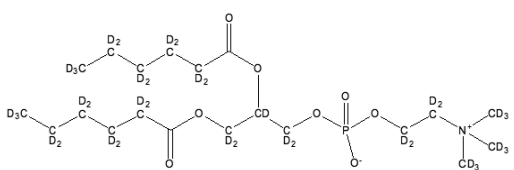
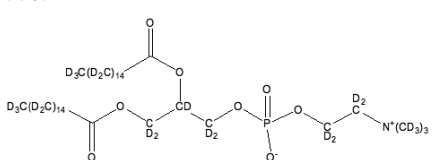
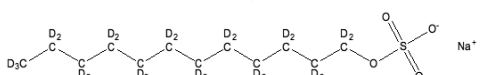
Membrane proteins can be divided into three categories:

1. Integral membrane proteins, which penetrate the lipid bilayer;
2. Peripheral membrane proteins, which are external and bound through noncovalent interactions; and
3. Lipid-anchored proteins, which are external, but bound with covalent bonds.

There is great interest in determining structure of integral membrane proteins due to the importance of these proteins in participating in cellular processes. Despite the significant functional importance of membrane proteins, the structural biology has been particularly challenging.

The determination of structure and dynamics of membrane proteins using NMR requires samples containing protein that is properly folded. Fortunately, membrane proteins often keep native-like structures in detergent micelles. Deuterated solubilization agents such as detergents often make NMR investigations easier compared to using unlabeled agents. In some cases, as in methyl labeling, deuterated reagents of this type are required. CIL is pleased to offer deuterated detergents and phospholipid agents for use with membrane proteins.

### Deuterated Detergents and Phospholipids

Catalog No.	Description
DLM-2274	Dodecylphosphocholine ( $D_{38}$ , 98%)
	
DLM-6726	N-Octyl $\beta$ -Glucoside ( $D_{24}$ , 98%)
	
DLM-4341	DL-A-Phosphatidylcholine, dihexanoyl ( $D_{40}$ , 98%) (DHPC) CP 95%
	
DLM-8256	DL-A-Phosphatidylcholine, dipalmitoyl (DPPC) ( $D_{80}$ , 98%) CP 95%+
	
DLM-197	Sodium dodecyl sulfate ( $D_{25}$ , 98%)
	

“The methodological development that NMR has seen in the past six decades would not have been possible without close collaborations with companies that love to see progress happening. CIL is a particularly strong and supportive partner, and many ideas with respect to labeling patterns that looked crazy on the first sight became reality through CIL, enabling us to conquer new areas for NMR structure determination. There is a wide variety of examples where talking to CIL has pushed science forward.”

Hartmut Oschkinat, PhD  
Department of NMR – Supported Structural Biology  
FMP Berlin

## The Magic of MAS DNP

Robert Guy Griffin, PhD; Kong Ooi Tan, PhD; Brian C. Michael

Francis Bitter Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA USA

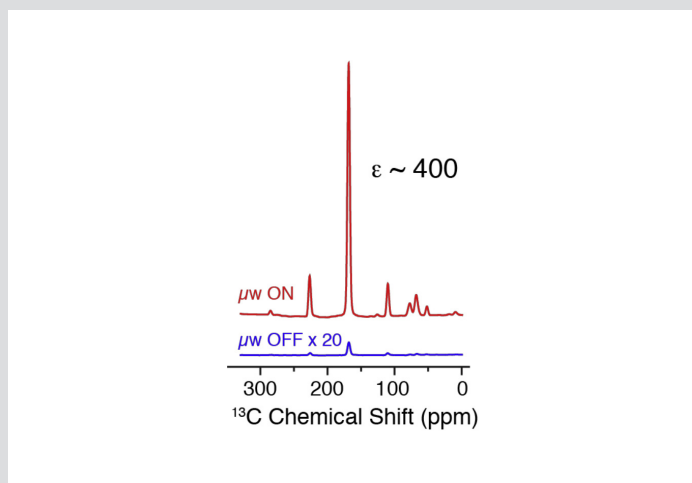
Investigator  
Spotlight

Dynamic nuclear polarization (DNP)<sup>1</sup> is a hyperpolarization technique that enhances the sensitivity of NMR by transferring polarization from electrons to nuclei with a theoretical maximum enhancement factor of up to 658 when the nucleus is <sup>1</sup>H. In a DNP sample, paramagnetic species such as stable organic radicals<sup>2-5</sup> are dispersed into a glassy matrix containing the sample of interest, which is then studied in magic angle spinning (MAS) probes capable of operating at cryogenic temperatures (< 110 K). The key instrumentation that enables MAS-DNP at high fields is the gyrotron, which provides the high-power microwave irradiation needed for DNP experiments. The use of gyrotrons for DNP was developed here at MIT and later made commercially available from Bruker.<sup>6,7</sup>

Sensitivity enhancements from DNP have allowed us to study important biological samples such as amyloid fibrils<sup>8</sup> and membrane proteins<sup>9</sup> with NMR experiments that are otherwise not feasible within a reasonable timeframe. For instance, **Figure 1** shows a spectrum of <sup>13</sup>C/<sup>15</sup>N-labeled urea with a ~400-fold signal enhancement from DNP.<sup>10</sup> In other words, the DNP-enhanced experiment performed in an hour would have taken ~18 years without DNP to obtain the spectrum with the same signal-to-noise ratio.

In order to remain intact under the conditions used in DNP experiments, biological samples usually need to be cryoprotected in a matrix that forms a glass at low temperatures. This matrix is typically a 6:3:1 mixture of *d*<sub>8</sub>-glycerol, D<sub>2</sub>O, and H<sub>2</sub>O, which is universally known as “DNP juice.” The matrix can be further optimized by using deuterated <sup>13</sup>C-depleted glycerol to suppress unwanted <sup>13</sup>C background signals. Additionally, partial deuteration of biological samples has been shown to improve DNP enhancements.<sup>11</sup> <sup>2</sup>H/<sup>13</sup>C/<sup>15</sup>N-labeled samples can be prepared by expressing proteins and peptides in *E. coli* in a D<sub>2</sub>O-based medium using <sup>15</sup>N-ammonium chloride, and <sup>2</sup>H/<sup>13</sup>C-glucose. We have also made use of a variety of sparsely labeled samples prepared using selectively <sup>13</sup>C-labeled 1,6-<sup>13</sup>C<sub>2</sub>-glucose or 2-<sup>13</sup>C- and 1,3-<sup>13</sup>C<sub>2</sub>-glycerol.

DNP has also been applied to a variety of other problems, such as pharmaceutical products<sup>12</sup> and inorganic materials.<sup>13</sup> While MAS-NMR studies make extensive use of <sup>1</sup>H, <sup>15</sup>N, <sup>13</sup>C, and even <sup>31</sup>P, very little work has been done on <sup>17</sup>O despite its high abundance in biological systems. Historically, <sup>17</sup>O studies have been limited, because the NMR-active isotope, <sup>17</sup>O, has a large quadrupolar coupling and low sensitivity. We are currently developing <sup>17</sup>O-based experiments for use at higher magnetic fields and in conjunction with improved sensitivity from DNP.<sup>14,15</sup> We foresee continued development of <sup>17</sup>O spectroscopy which should enable the extraction of rich information from biological samples in the future.



**Figure 1.** MAS-DNP on <sup>13</sup>C/<sup>15</sup>N urea in “DNP juice.”

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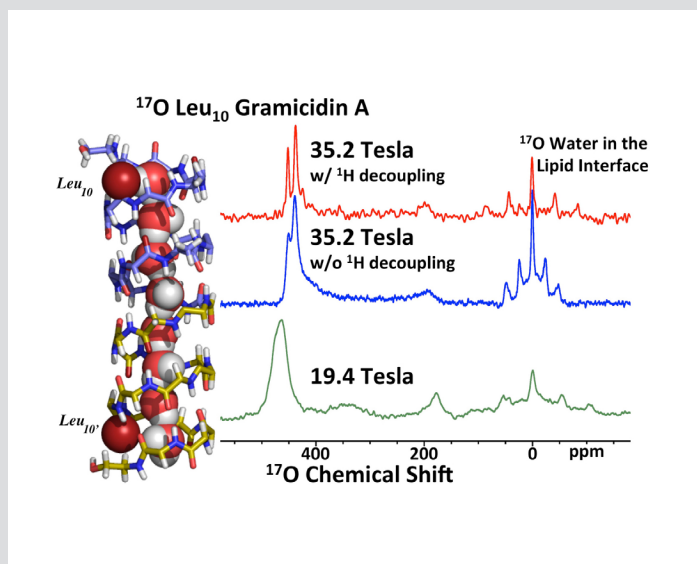
## $^{17}\text{O}$ Solid State NMR Spectroscopy: Coming of Age

Investigator  
Spotlight**Timothy A. Cross, PhD**NMR and MRI User Program Director, National High Field Magnetic Lab  
Chemistry and Biochemistry Professor, Florida State University, Tallahassee, Florida USA

High fields bring added sensitivity and spectral dispersion for NMR, but the influence of high fields is much greater for quadrupolar nuclei than for our commonly studied spin  $\frac{1}{2}$  nuclei.<sup>1</sup> At the National High Magnetic Field Lab we have recently commissioned a powered magnet for NMR operating at 35.2 Tesla or 1500 MHz.<sup>2</sup> As a powered magnet it has to be ramped to field (~25 min) when we need it and ramped down as soon as an experiment is completed. We have incorporated  $^{17}\text{O}$ -labeled glycine and leucine into the antibiotic, gramicidin A (gA), a 15-amino-acid peptide that as a dimer forms a monovalent cation selective channel.<sup>3,4</sup> The structure is an alternating sequence of D and L amino acids that as a  $\beta$ -strand wraps up into a helix of 6.3 residues per turn having a pore that supports a single file column of eight water molecules.<sup>3,5</sup> These waters are hydrogen bonded together forming what is known as a water wire (Figure 1) in a space-filling view inside the pore formed by the gA dimer that spans cellular membranes.

The  $^{17}\text{O}$  solid state NMR (ssNMR) spectra are dramatically enhanced at higher magnetic fields both in sensitivity and in linewidth.<sup>6</sup> Here, at 35.2 T the reduction in the  $^{17}\text{O}$  linewidth of oriented sample (OS) ssNMR resonances is such that  $^1\text{H}$  decoupling makes a further dramatic difference (Figure 1) leading to the observation of a pair of resonances for a single site-labeled sample of gA,<sup>7</sup> something we did not observe when working with spectra at 19.4 and 21.1 T.<sup>8</sup> The  $^{17}\text{O}$  studies in superconducting failed to observe a resolvable difference in the spectra of single site  $^{17}\text{O}$ -labeled gA. In hindsight there was an asymmetry in the resonance, but we did not anticipate that the sites in the two monomers could be distinguished after all the high-resolution  $^{15}\text{N}$  OS ssNMR spectra of the gA backbone showed no evidence of two resonances when a single site was labeled.<sup>2,4</sup> With  $^1\text{H}$  decoupling and exceptional sensitivity the  $^{17}\text{O}$  spectroscopy at this field strength provided a tool that is known to be exceptionally sensitive to hydrogen bonding.<sup>9-11</sup> It became clear that since the structure of the two monomers were identical the only possible distinction between the two monomers was the hydrogen bonding from the waters to the carbonyl oxygens lining the pore of the channel.<sup>7</sup>

**With  $^1\text{H}$  decoupling and exceptional sensitivity the  $^{17}\text{O}$  spectroscopy at this field strength provided a tool that is known to be exceptionally sensitive to hydrogen bonding.**



**Figure 1.** The  $^{17}\text{O}$  OS NMR spectra of  $\text{Leu}_{10}$ -labeled gA in liquid crystalline lipid bilayers as a function of field strength. Note that the carbonyl oxygen labeled  $\text{Leu}_{10}$  (dark red) is hydrogen bonded to a water proton (white spheres), but  $\text{Leu}_{10}$  in the second monomer is not hydrogen bonded to a water proton, generating a 15 ppm chemical shift difference for the two resonances.<sup>7</sup>

Between the two  $\text{Leu}_{10}$  residues are 24 carbonyl oxygens and there are just eight water molecules that have two protons each, but one of the protons is required in forming the water wire, as a result there are only eight protons that could potentially hydrogen bond to a selection of the 24 carbonyl oxygens. The water wire means that the waters are all oriented in the same direction from one side of the membrane to the other, but the gA monomers are oriented in an antiparallel fashion with both of their amino termini at the center of the bilayer. Consequently, the interactions of the waters in one monomer are different from the interactions of the waters with the other monomer. This results in a unique pair of resonances for each site within the water wire region of the pore.<sup>7</sup>

In addition to these  $^{17}\text{O}$  OS ssNMR experiments, Griffin and coworkers have been working on the development of  $^{17}\text{O}$  magic angle spinning ssNMR spectroscopy at 35.2 T<sup>12,13</sup> – those interested in high-field spectroscopy should get in touch with the NMR/MRI User program at the NHMFL to apply for access to the 35.2 T spectrometer available without any user fees, thanks to NSF.



## <sup>17</sup>O Solid State NMR Spectroscopy: Coming of Age (continued)

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## <sup>17</sup>O NMR Reagents

Even though oxygen is ubiquitous to all lifeforms, the use of <sup>17</sup>O NMR to study biological molecules is rare, due to the low natural abundance of the <sup>17</sup>O isotope (e.g., 0.037%) and unfavorable NMR properties of the <sup>17</sup>O nucleus (low sensitivity and very broad resonances). Thus, molecules generally require enrichment using <sup>17</sup>O-containing starting materials (e.g., H<sub>2</sub><sup>17</sup>O) for analysis using <sup>17</sup>O NMR. Despite these constraints, <sup>17</sup>O NMR encompasses a wide chemical shift range (~1650 ppm) and therefore holds much promise in gaining new insight in determining structure and the chemical behavior of biological molecules.

There has been much progress in utilizing <sup>17</sup>O NMR to study biological molecules. For example, the laboratory of Dr. Gang Wu (Queens University, Ontario, Canada) reported the use of Quadrupole Central Transition (QCT) <sup>17</sup>O NMR spectroscopy to probe large protein-ligand complexes in solution.<sup>1</sup> They demonstrated that <sup>17</sup>O-labeled ligands at ~30% <sup>17</sup>O enrichment can be detected in protein-ligand complexes of 240 kDa with <1 mM protein concentrations, but expect the technique to work with complexes as large as 400-500 kDa when using <sup>17</sup>O enrichments as high as 90%. They also reported QCT <sup>17</sup>O NMR spectroscopy should be applicable to a wide variety of biological macromolecules. Additionally, this laboratory reported the first comprehensive solid-state <sup>17</sup>O MAS NMR of large protein-ligand complexes.<sup>2</sup>

CIL is pleased to offer highly enriched <sup>17</sup>O water as a starting material to probe protein-ligand complexes using either QCT <sup>17</sup>O NMR or solid-state <sup>17</sup>O MAS NMR. Additionally, mechanochemistry has been shown to be a well-suited technique for <sup>17</sup>O labeling of organic and inorganic compounds.<sup>3</sup>

Catalog No.	Description
OLM-782-90	Water ( <sup>17</sup> O, 90%)
OLM-782-85	Water ( <sup>17</sup> O, 85%)
OLM-782-70	Water ( <sup>17</sup> O, 70%)
OLM-782-60	Water ( <sup>17</sup> O, 60%)
OLM-782-40	Water ( <sup>17</sup> O, 35-40%)
OLM-782-20	Water ( <sup>17</sup> O, 20%)
OLM-782-10	Water ( <sup>17</sup> O, 10%)
NOLM-10743	L-Tyrosine ( <sup>15</sup> N, 98%; carboxyl- <sup>17</sup> O <sub>2</sub> , 60%)
NOLM-10742	L-Valine ( <sup>15</sup> N, 98%; carboxyl- <sup>17</sup> O <sub>2</sub> , 60%)

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3. Metro, T.X.; Gervais, C.; Martinez, A.; et al. **2017**. Unleashing the potential of <sup>17</sup>O NMR spectroscopy using mechanochemistry. *Angew Chem Int Ed*, 56(24), 6803-6807.

## Miscellaneous Protein Reagents

### Reverse Micelles

NMR spectroscopy of encapsulated proteins dissolved in low viscosity fluids has emerged as a powerful supplement to traditional solution NMR approaches. Originally developed to overcome the slow tumbling problem presented by large soluble proteins,<sup>1</sup> the general approach of using reverse micelles has now seen applications in the study of integral<sup>2</sup> and anchored<sup>3</sup> membrane proteins; proteins of marginal stability;<sup>4</sup> protein structure,<sup>5</sup> dynamics<sup>6</sup> and hydration.<sup>7</sup> Nucleic acids have also been successfully investigated in this manner.<sup>8</sup> The distinguishing feature of this approach is the nature of the sample. Spontaneously formed reverse micelles are the dominant vehicle for encapsulation and the low-viscosity short-chain alkane fluids are the dominant solvent with liquid propane and ethane being the most desirable.<sup>9</sup> The availability of deuterated surfactants, cosurfactants and alkane solvents avoids the complications of large unwanted <sup>1</sup>H resonances that would greatly interfere with multidimensional NMR of encapsulated biopolymers in low-viscosity solvents.

### References

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- Peterson, R.W.; Anbalagan, K.; Tommos, C.; et al. **2004**. Forced folding and structural analysis of metastable proteins. *J Am Chem Soc*, 126(31), 9498-9499.
- Babu, C.R.; Flynn, P.F.; Wand, A.J. **2001**. Validation of protein structure from preparations of encapsulated proteins dissolved in low-viscosity fluids. *J Am Chem Soc*, 123(11), 2691-2692.
- Simorellis, A.K.; Flynn, P.F. **2006**. Fast local backbone dynamics of encapsulated ubiquitin. *J Am Chem Soc*, 128(30), 9580-9581.
- Nucci, N.V.; Pometun, M.S.; Wand, A.J. **2011**. Site-resolved measurement of water-protein interactions by solution NMR. *Nat Struct Mol Biol*, 18(2), 245-249.
- Workman, H.; Flynn, P.F. **2009**. Stabilization of RNA oligomers through reverse micelle encapsulation. *J Am Chem Soc*, 131(11), 3806-3807.
- Peterson, R.W.; Lefebvre, B.G.; Wand, A.J. **2005**. High-resolution NMR studies of encapsulated proteins in liquid ethane. *J Am Chem Soc*, 127(29), 10176-10177.

### Reagents for Reverse Micelles

Catalog No.	Description
DLM-276	Ethane (D <sub>6</sub> , 98%)
DLM-8117	Hexadecyltrimethylammonium bromide (D <sub>42</sub> , 98%)
DLM-8840	Hexadecyltrimethylammonium chloride (D <sub>42</sub> , 98%)
DLM-691	<i>n</i> -Hexanol (D <sub>13</sub> , 98%)
DLM-1213	<i>n</i> -Pentane-D <sub>12</sub> (D, 98%)
DLM-3476	Propane (1,1,1,3,3,3-D <sub>6</sub> , 98%)

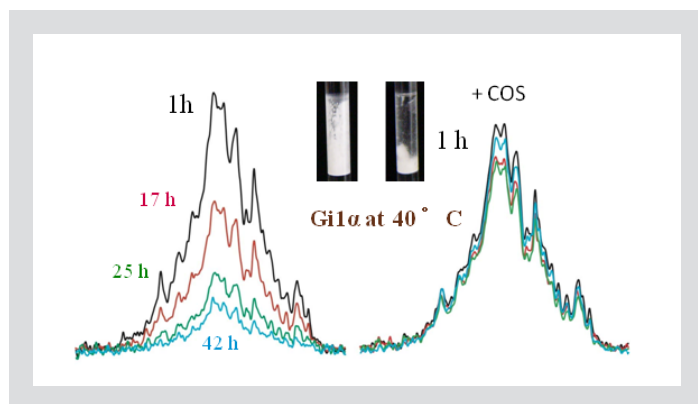
### Deuterated Reagents

Catalog No.	Description
DLM-710	Ammonium deuterioxide (D <sub>5</sub> , 99%) (~25% ND <sub>4</sub> OD in D <sub>2</sub> O)
DLM-54	Deuterium chloride (D, 99.96%) (~20% DCl in D <sub>2</sub> O)
DLM-2	Deuterium chloride (D, 99.5%) (~20% DCl in D <sub>2</sub> O)
DLM-3	Deuterium chloride (D, 99.5%) (~35% DCl in D <sub>2</sub> O)
DLM-414	EDTA (D <sub>12</sub> , 98%)
DLM-3908	EDTA (D <sub>16</sub> , 98%)
DLM-2622	DL-1,4-Dithiothreitol (DTT) (D <sub>10</sub> , 98%)
DLM-6686	tris(2-Carboxyethyl)phosphine-DCl (TCEP) (D <sub>16</sub> , 98%)
DLM-2713	2-Mercaptoethanol (D <sub>6</sub> , 98%)

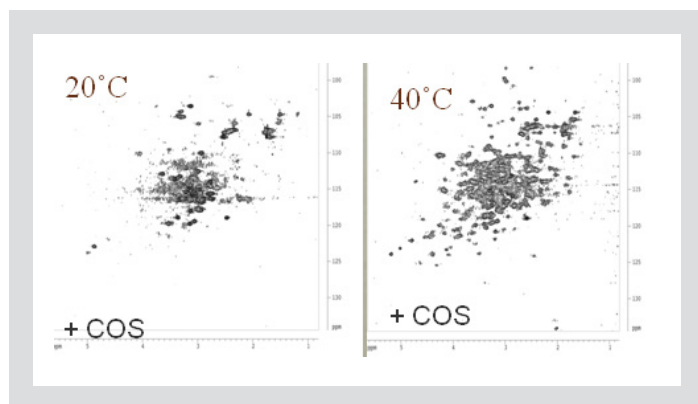
## Miscellaneous Protein Reagents

### Protein Stabilization Reagent

Dr. Kaori Wakamatsu from Gunma University (Gunma, Japan) has pioneered the use of choline-*O*-sulfate (COS) and uniformly deuterated choline-*O*-sulfate (COS-D<sub>13</sub>) for use in protein NMR studies. His results show significant improvement in stabilizing soluble forms of protein in solution with a concurrent increase in sensitivity in multidimensional NMR data sets acquired at near-human physiological temperatures.



**Figure 1.** 1D HSQC spectra of uniformly <sup>15</sup>N-labeled Gi1α in the absence (left) or presence of 1 M COS (right) were recorded on a Bruker ARX-400 spectrometer at 40°C at 1 h (black), 17 h (red), 25 h (green), and 42 h (cyan) after temperature control. The photographs demonstrate much less precipitate in the presence of COS at 42 h.



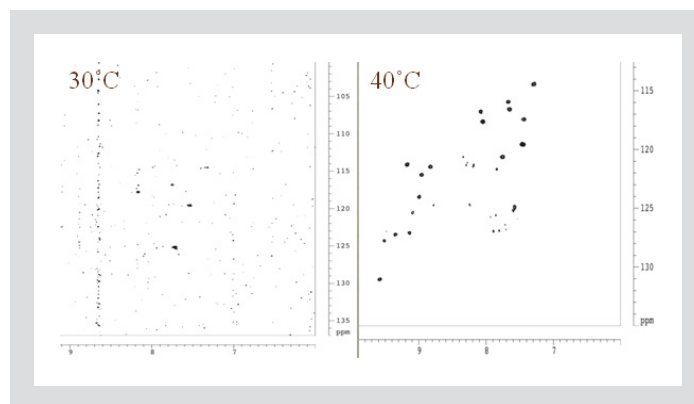
**Figure 2.** HSQC spectra of uniformly <sup>15</sup>N labeled Gi1α in the presence of 1 M COS at 20°C (left) and 40°C (right). Spectra were recorded on a Bruker ARX-400 spectrometer.

### Advantages of COS:

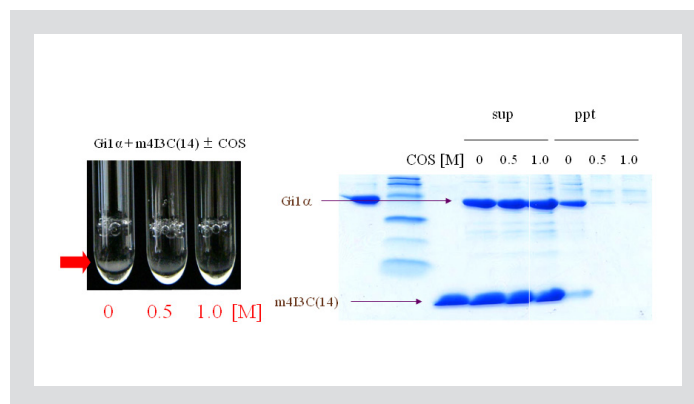
- Prevention of thermal denaturation of membrane proteins, including GPCRs
- Prevention of precipitation of protein and protein/peptide complexes
- Facilitation of NMR measurements, especially at elevated temperatures
- Improvement of protein recovery during purification

CIL is pleased to offer unlabeled and uniformly deuterated COS to the worldwide protein NMR community.

Catalog No.	Description
DLM-8937	Choline- <i>O</i> -sulfate (D <sub>13</sub> , 98%)
ULM-8703	Choline- <i>O</i> -sulfate (unlabeled)



**Figure 3.** HSQC spectra of Gi1α labeled with [<sup>15</sup>N] phenylalanine in the presence of 1 M COS-d<sub>13</sub> at 30°C (left) and 40°C (right). Almost all (18 out of 19) phenylalanine signals are clearly observed at 40°C. Spectra were recorded on a Bruker Avance-700 spectrometer.



**Figure 4.** COS prevents co-precipitation of Gi1α and its selective activator, m4B3C(14), on mixing. Gi1α and m4B3C(14) form precipitates on mixing (photograph at bottom, left tube). The composition of the precipitates is confirmed by SDS-PAGE (bottom right panel, lane ppt/0). In the presence of COS, the precipitates are not observed (photograph, SDS-PAGE).

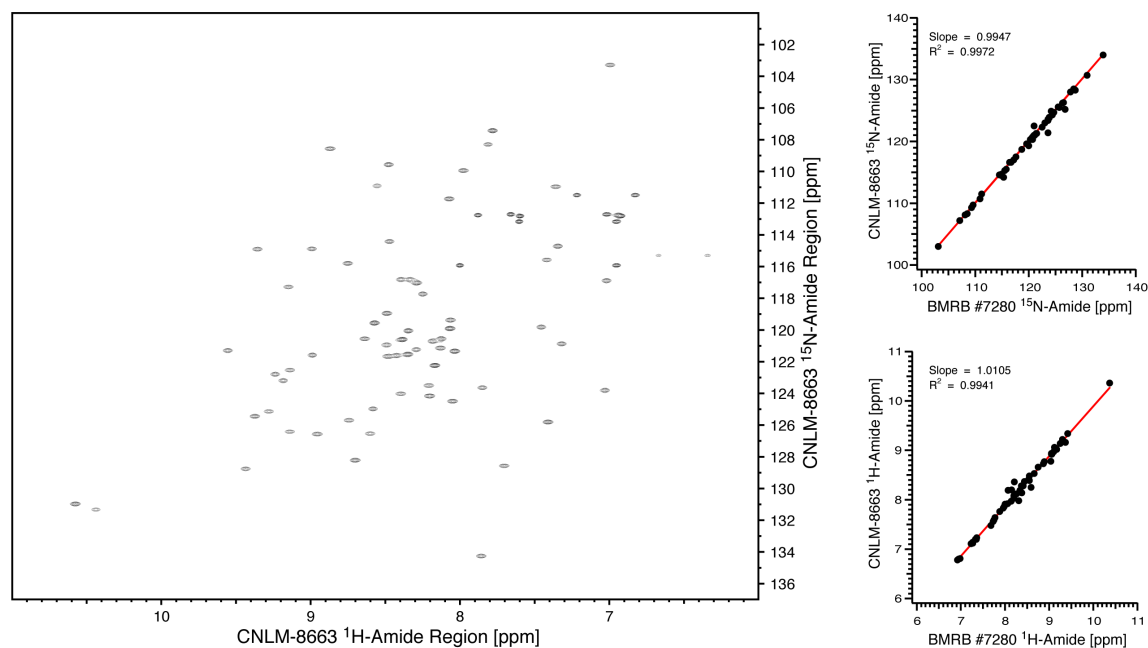
## Protein NMR Standards

CIL offers labeled NMR standards that are used to test new pulse sequences and monitor spectrometer performance.

Catalog No.	Description
CNLM-2408	GFL Peptide Standard ( $^{13}\text{C}$ , 98%; $^{15}\text{N}$ , 96-99%) 1 mM in DMSO- $d_6$
<b>Amino Acid Sequence</b> YG <b>GFL</b> RR (bold indicates labeled residues)	
CNLM-8663	His-GB1 ( $^{13}\text{C}$ , 98%; $^{15}\text{N}$ , 98%) 1.5 mM in PBS, pH 6.5, 0.02% sodium azide, 0.1 mM DSS, 10% $\text{D}_2\text{O}$
<b>Amino Acid Sequence</b> MHHHHHHGEN LYFQSMQYKL ILNGKTLKGE TTTEAVDAAT AEKVKFYAN DNGVDGEWTD DDAKTFTVT E	

Also available in prepackaged 5 mM NMR tube; please inquire.

**Don't have the time or resources to express your desired protein?** Ask your local CIL sales representative about the possibility of supplying you with uniformly labeled, intact protein. Specify the following information: complete protein sequence; type and location of a purification tag (if desired); labeling scheme; level of chemical purity; and desired amount (in milligrams).



$^1\text{H}$ ,  $^{15}\text{N}$ -HSQC of 1.5 mM immunoglobulin-binding fomain B1 of streptococcal protein G ( $^{13}\text{C}$ , 99%;  $^{15}\text{N}$ , 99%) containing an *N*-terminal his6-tag and tobacco etch virus protease (TEV) cleavage site (catalog no. CNLM-8663-CA, Lot #20110209). The  $^{15}\text{N}$ -amide (top, right) and  $^1\text{H}$ -amide (bottom, right) assignments of CNLM-8663-CA show excellent correlation with those previously reported in the Biological Magnetic Resonance Bank for GB1(BMRB #7280) lacking the his6-TEV leader sequence.

## Protein Standards from Nexomics Biosciences, Inc.

CIL is pleased to offer Nexomics Biosciences, Inc. isotope-enriched proteins for use as standards in NMR spectroscopy. Isotope-enriched protein standards are ideal for:

- Aiding in the development of new pulse sequences
- Optimizing parameters for a given pulse sequence
- Assessing spectrometer performance
- Training purposes

Standards are available with protein concentrations of 0.25 mM, 0.5 mM, and 1 mM. Please specify preferred concentration. Standard packaging in an Eppendorf tube; 3 mM and 5 mM NMR tubes are available upon request.

Nexomics is a New Jersey-based contract research organization that specializes in a broad array of gene-to-structure services to

the biopharmaceutical community. Nexomics provides high-quality, high-purity standards that are invaluable tools for bioNMR. Each product is accompanied by the following data:

- $^1\text{H}$ - $^{15}\text{N}$  HSQC ( $^{15}\text{N}$ -labeled proteins)
- $^1\text{H}$ - $^{13}\text{C}$  HSQC ( $^{13}\text{C}$ -labeled proteins)
- CO-NH projection of 3D HNCO ( $^{15}\text{N}$ ,  $^{13}\text{C}$ -labeled proteins)
- SDS PAGE (for all labeled proteins)
- MALDI-TOF (for all labeled proteins)
- $^{15}\text{N}$ -edited X-filtered 2D NOESY (NEX-XF1)



### Maltose-Binding Periplasmic Protein (MBP) from *E. coli* (strain K12) (residues 27-396)

NEX-MBP is a 40.84 kDa monomeric protein for which multiple sets of resonance assignments (BMRB database) and 3D structures (PDB database) are publicly available. As nonuniform sampling (NUS) and other NMR techniques emerge to push the size limitations of NMR to new boundaries, large protein standards, such as NEX-MBP, will be required to test data-collection and processing strategies.

#### NEX-MBP1: Apo Conformation

**Formulation:** 20 mM sodium phosphate buffer (pH 7.2) in 10%  $\text{D}_2\text{O}$  and 0.02%  $\text{NaN}_3$

**Number of residues:** 370

**MW (unlabeled):** 40.84 kDa

**Theoretical pI:** 5.2

**UniProt Accession:** P0AEX9

Catalog No.	Label
NEX-MBP1-U	unlabeled
NEX-MBP1-N	( $^{15}\text{N}$ , 95%)
NEX-MBP1-CN-5	( $^{13}\text{C}$ , 5%; $^{15}\text{N}$ , 95%)
NEX-MBP1-CN	( $^{13}\text{C}$ , 95%; $^{15}\text{N}$ , 95%)
NEX-MBP1-CDN	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%)
NEX-MBP1-ILV	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV)
NEX-MBP1-ILVFI	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILVFI)

#### NEX-MBP2: Closed Conformation

**Formulation:** 3 mM maltotriose, 20 mM sodium phosphate buffer (pH 7.2) in 10%  $\text{D}_2\text{O}$  and 0.02%  $\text{NaN}_3$

**Number of residues:** 370

**MW (unlabeled):** 40.84 kDa

**Theoretical pI:** 5.2

**UniProt Accession:** P0AEX9

Catalog No.	Label
NEX-MBP2-U	unlabeled
NEX-MBP2-N	( $^{15}\text{N}$ , 95%)
NEX-MBP2-CN-5	( $^{13}\text{C}$ , 5%; $^{15}\text{N}$ , 95%)
NEX-MBP2-CN	( $^{13}\text{C}$ , 95%; $^{15}\text{N}$ , 95%)
NEX-MBP2-CDN	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%)
NEX-MBP2-ILV	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV)
NEX-MBP2-ILVFI	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILVFI)

#### NEX-MBP3: Open Conformation

**Formulation:** 2 mM  $\beta$ -cyclodextrin, 20 mM sodium phosphate buffer (pH 7.2) in 10%  $\text{D}_2\text{O}$  and 0.02%  $\text{NaN}_3$

**Number of residues:** 370

**MW (unlabeled):** 40.84 kDa

**Theoretical pI:** 5.2

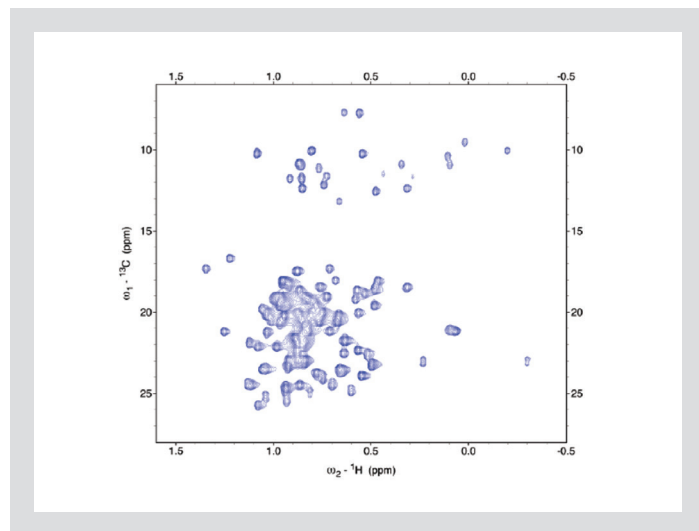
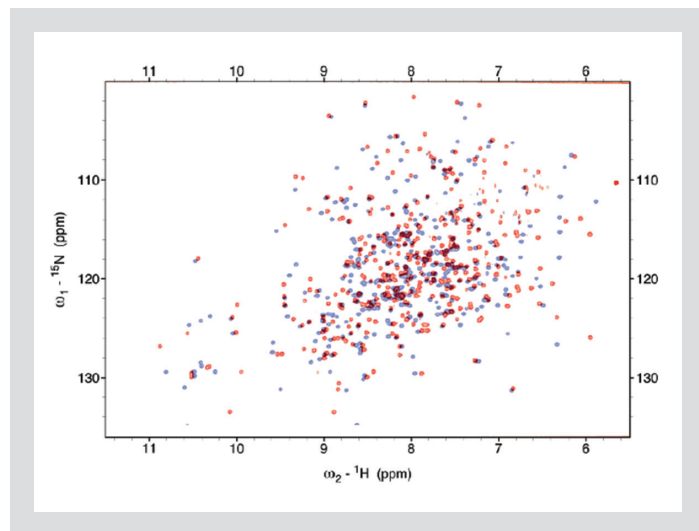
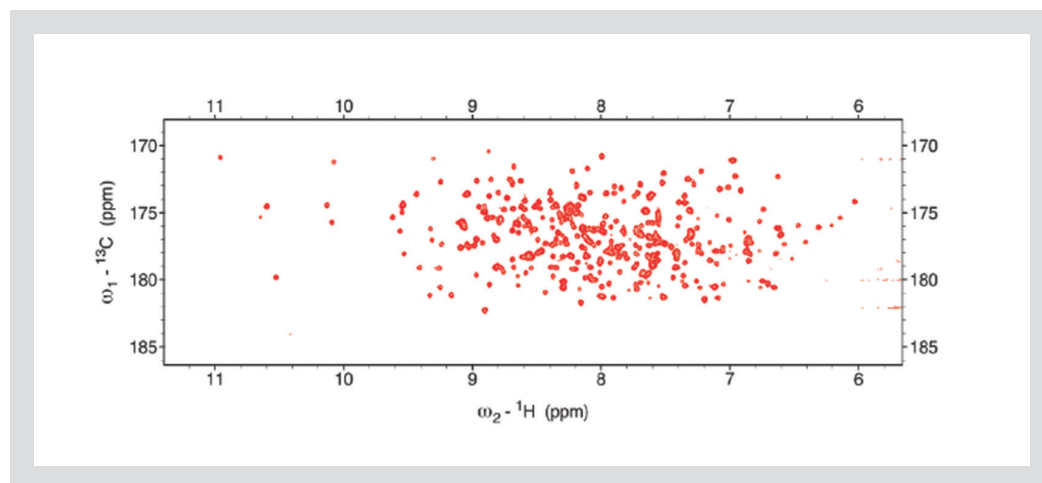
**UniProt Accession:** P0AEX9

Catalog No.	Label
NEX-MBP3-U	unlabeled
NEX-MBP3-N	( $^{15}\text{N}$ , 95%)
NEX-MBP3-CN-5	( $^{13}\text{C}$ , 5%; $^{15}\text{N}$ , 95%)
NEX-MBP3-CN	( $^{13}\text{C}$ , 95%; $^{15}\text{N}$ , 95%)
NEX-MBP3-CDN	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%)
NEX-MBP3-ILV	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV)
NEX-MBP3-ILVFI	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILVFI)

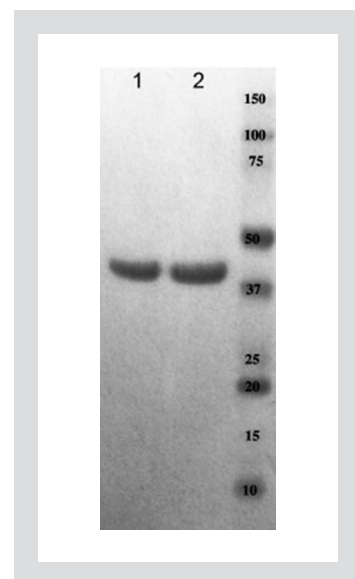
#### Protein Sequence

MKIEEGKLVWINGDKGYNGLAIEVGKKFEKDTGKIVTEHPDKLEEFQVAATGDGPDIIIFWAH  
 DRFGGYAQSGLLAEITPDKAFQDKLPFTWDVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEE  
 IPALDKELKAKGKSALMFNLQEPYFTWPLIADGGYAFKYENGKYDIKDVGDVGNAGAKAGLTL  
 VDLIKNKHMNADTDYSIAEAFNKGETAMTINGPWAWSNIDTSKVNYGTVLPTFKGQPSKP  
 FVGVLGAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMEN  
 AQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQTRITK

## Protein Standards from Nexomics Biosciences, Inc. (continued)

 $^{13}\text{C}$ ,  $^1\text{H}$  HSQC NEX-MBP3 "open" conformation. $^{13}\text{C}$ ,  $^1\text{H}$  HSQC "open" (blue) and "closed" (red) superposition.

CO-NH 2D plane of HNCO triple-resonance experiment of NEX-MBP2 "closed" sample.

SDS-PAGE GEL NEX-MBP (right). NEX-MBP3  $\beta$ -cyclodextrin complexed "open" sample (lane 1). NEX-MBP2 maltotriose complexed "closed" sample (lane 2).



## X-Filtered NOESY NMR Standard (NEX-XF1)

In an X-filtered experiment, only NOEs between  $^{15}\text{N}/^{13}\text{C}$ - $^1\text{H}$  and  $^{14}\text{N}/^{12}\text{C}$ - $^1\text{H}$  (e.g., interchain NOEs) protons are observed. NOEs between protons connected to  $^{15}\text{N}, ^{13}\text{C}$  are filtered (intrachain NOEs). When uniformly double-labeled protein sample is mixed with a natural-abundance protein sample, the interface will give rise to the only observable NOESY cross peaks. This powerful strategy enables the spectroscopist to discern intra from inter NOESY cross peaks, thereby providing essential distance constraints for defining the dimer interface (Lee, et al., 1994, 350, 87; Palmer, et al., 1991, 93, 151; Schleucher, et al., 1994, 4, 301).

NEX-XF1 is a 10.95 kDa protein (*A. fulgidus* antitoxin vapB21 homodimer) for which a set of resonance assignments (bmr7362), 3D structure (2NWT) and other NMR data are available in the public domain. This is a mixture of unlabeled and uniformly  $^{15}\text{N}/^{13}\text{C}$ -enriched protein (25% homodimer unlabeled; 50% heterodimer unlabeled/labeled; 25% homodimer labeled) and is perfect to set up X-filtered NOESY experiments.

**NEX-XF1:** Mixture of 25% homodimer unlabeled; 50% heterodimer unlabeled/labeled; 25% homodimer labeled

**Formulation:** 20 mM  $\text{NH}_4\text{OAc}$  (pH 5.5), 100 mM NaCl, 5 mM  $\text{CaCl}_2$ , 10%  $\text{D}_2\text{O}$ , 0.02 %  $\text{NaN}_3$

**Number of residues:** 38

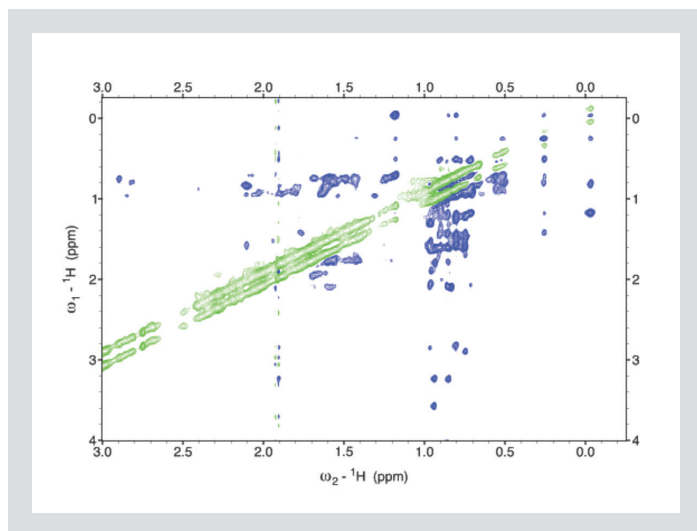
**MW (unlabeled):** 10.95 kDa (homodimer)

**Theoretical pI:** 7.0

**UniProt Accession:** O28071

## X-Filtered NOESY NMR Standard, His-Tagged

Catalog No.	Label
NEX-XF1-HIS-CN	( $^{13}\text{C}$ , 95%; $^{15}\text{N}$ , 95%)

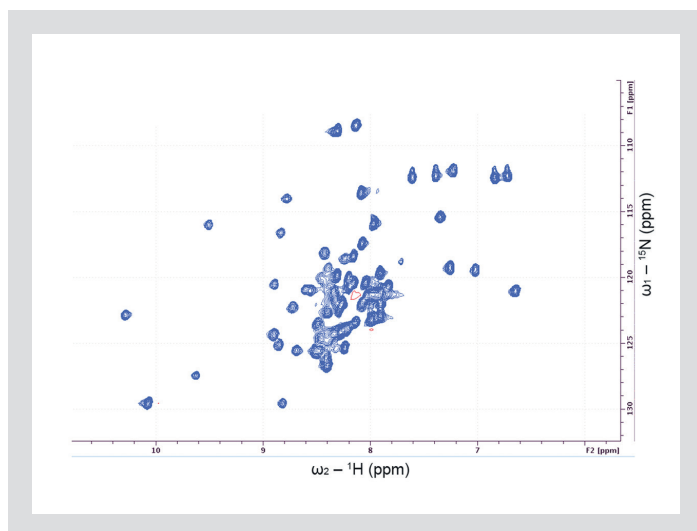
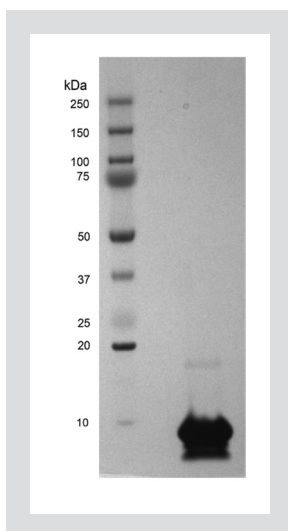


2D  $^1\text{H}$ - $^1\text{H}$  plane of  $^1\text{H}$ ,  $^{13}\text{C}$  edited  $^1\text{H}$ ,  $^{12}\text{C}$  X-filtered NOESY.

## Protein Sequence

MPKVYIYENGVFQKQKVDLKEGERVKIKLELKVE  
PLEHHHHHH

SDS-PAGE GEL NEX-XF1 (right).



$^1\text{H}$ - $^{15}\text{N}$  HSQC of NEX-XF1.

## Protein Standards from Nexomics Biosciences, Inc. (continued)

## Human Ubiquitin-60S Ribosomal Protein L40 (residues 1-76) (NEX-UB1)

NEX-UB1 is a small 8.8 kDa monomeric protein for which multiple sets of resonance assignments (BMRB database) and 3D structures (PDB database) are publicly available. Ubiquitin has been used as an industry-wide standard in the protein NMR field for many years.

**NEX-UB1:** After TEV cleavage

**Formulation:** 10 mM sodium phosphate buffer (pH 6.5) in 10% D<sub>2</sub>O, 0.02 % NaN<sub>3</sub>

**Number of residues:** 76

**MW (unlabeled):** 8.79 kDa

**Theoretical pI:** 6.7

**UniProt Accession:** P62987

Catalog No.	Label
NEX-UB1-U	unlabeled
NEX-UB1-N	( <sup>15</sup> N, 95%)
NEX-UB1-CN-5	( <sup>13</sup> C, 5%; <sup>15</sup> N, 95%)
NEX-UB1-CN	( <sup>13</sup> C, 95%; <sup>15</sup> N, 95%)
NEX-UB1-CDN	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%)
NEX-UB1-ILV	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%; <sup>13</sup> CH <sub>3</sub> -ILV)
NEX-UB1-ILVfy	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%; <sup>13</sup> CH <sub>3</sub> -ILVfy)

**NEX-UB1-HIS:** With N-terminal His-Tag

**Formulation:** 10 mM sodium phosphate buffer (pH 6.5) in 10% D<sub>2</sub>O, 0.02 % NaN<sub>3</sub>

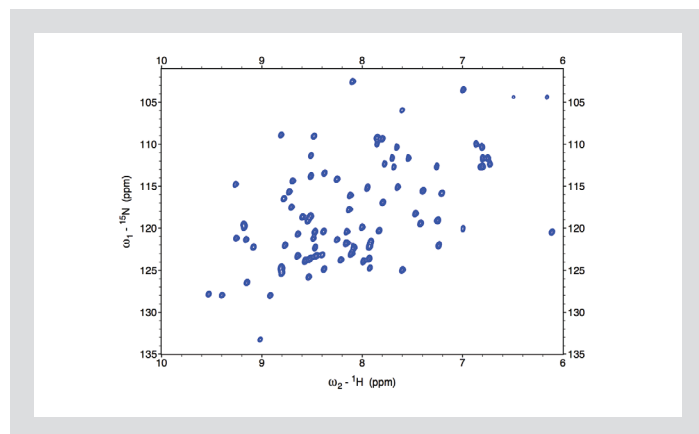
**Number of residues:** 90

**MW (unlabeled):** 10.59 kDa (with His-tag)

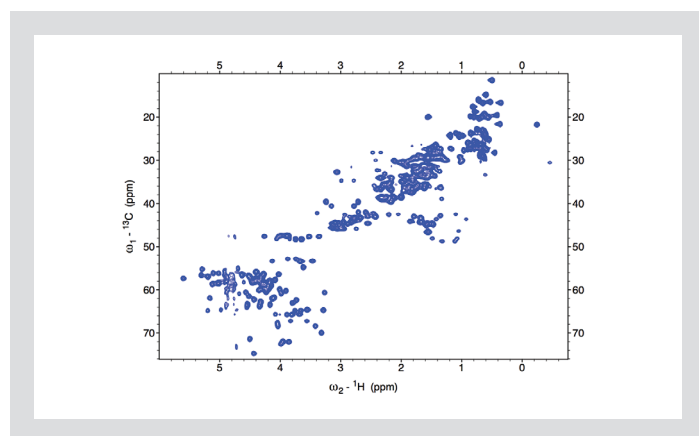
**Theoretical pI:** 6.7

**UniProt Accession:** P62987

Catalog No.	Label
NEX-UB1-HIS-U	unlabeled
NEX-UB1-HIS-N	( <sup>15</sup> N, 95%)
NEX-UB1-HIS-CN-5	( <sup>13</sup> C, 5%; <sup>15</sup> N, 95%)
NEX-UB1-HIS-CN	( <sup>13</sup> C, 95%; <sup>15</sup> N, 95%)
NEX-UB1-HIS-CDN	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%)
NEX-UB1-HIS-ILV	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%; <sup>13</sup> CH <sub>3</sub> -ILV)
NEX-UB1-HIS-ILVfy	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%; <sup>13</sup> CH <sub>3</sub> -ILVfy)



<sup>1</sup>H, <sup>15</sup>N HSQC of NEX-UB1.



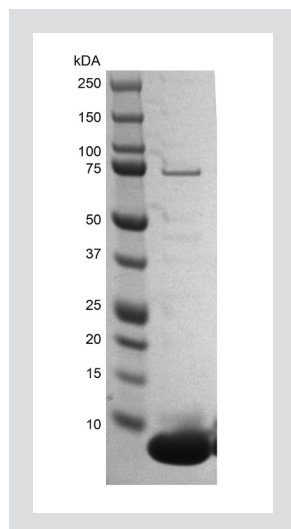
<sup>13</sup>C-<sup>1</sup>H HSQC of NEX-UB1.

### Protein Sequence after TEV Cleavage

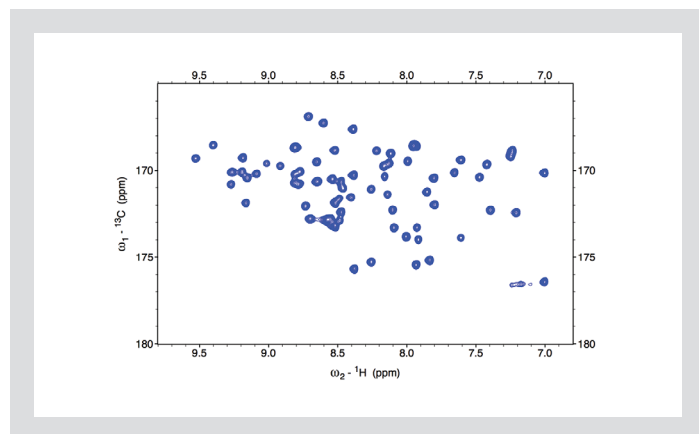
SHMQIFVKLTGKTTILEVPSDTIENVKAKIQD  
KEGIPPDQQR LIFAGKQLDGRTLSY  
NIQKESTLHLVLRRLGG

### Protein Sequence before TEV Cleavage

MGHHHHHHENLYFQSHMQIFVKLTGKTTI  
LEVPSDTIENVKAKIQDKEGIPPDQQR LIFAG  
KQLDGRTLSYNIQKESTLHLVLRRLGG



SDS-PAGE GEL NEX-UB1 (right).



CO-NH 2D plane of HNCO triple-resonance experiment of NEX-UB1.

## Immunoglobulin G-binding Protein G (GB1)

**Formulation:** 50 mM sodium phosphate (pH 5.5) in 10% D<sub>2</sub>O and 0.02% NaN<sub>3</sub>

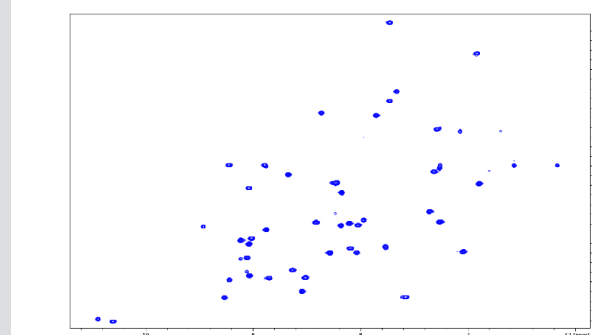
**Number of residues:** 56

**MW (unlabeled):** 6.22 kDa

**Theoretical pI:** 4.46

**UniProt Accession:** P06654

Catalog No.	Label
NEX-GB1-U	unlabeled
NEX-GB1-N	( <sup>15</sup> N, 95%)
NEX-GB1-CN-5	( <sup>13</sup> C, 5%; <sup>15</sup> N, 95%)
NEX-GB1-CN	( <sup>13</sup> C, 95%; <sup>15</sup> N, 95%)
NEX-GB1-CDN	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%)
NEX-GB1-ILV	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%; <sup>13</sup> CH <sub>3</sub> -ILV)
NEX-GB1-ILV FY	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%; <sup>13</sup> CH <sub>3</sub> -ILV FY)



### Protein Sequence

MQYKLILNGKTLKGETTTEAVDAATAEKVFKQYANDNGVDGEWTDATKTFVT

## Calbindin-D9k (Protein S100-G)

**Formulation:** 50 mM ammonium acetate (pH 6.0) in 10% D<sub>2</sub>O and 0.02% NaN<sub>3</sub>

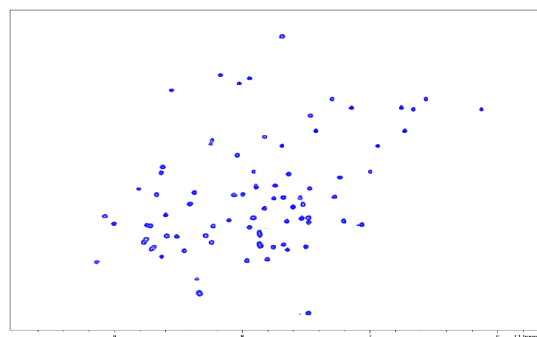
**Number of residues:** 76

**MW (unlabeled):** 8.63 kDa

**Theoretical pI:** 4.53

**UniProt Accession:** P02633

Catalog No.	Label
NEX-CAL-U	unlabeled
NEX-CAL-N	( <sup>15</sup> N, 95%)
NEX-CAL-CN-5	( <sup>13</sup> C, 5%; <sup>15</sup> N, 95%)
NEX-CAL-CN	( <sup>13</sup> C, 95%; <sup>15</sup> N, 95%)
NEX-CAL-CDN	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%)
NEX-CAL-ILV	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%; <sup>13</sup> CH <sub>3</sub> -ILV)
NEX-CAL-ILV FY	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%; <sup>13</sup> CH <sub>3</sub> -ILV FY)



### Protein Sequence

MKSPEELKGI FEKYAAKEGDPNQLSKEELKLLQTEFPSLLKGPSTLDELFEELDKNGDGEVSFEF  
QVLVKKISQ

## Alpha-Spectrin SH3 Domain

**Formulation:** 10 mM sodium citrate (pH 3.5) in 10% D<sub>2</sub>O and 0.02% NaN<sub>3</sub>

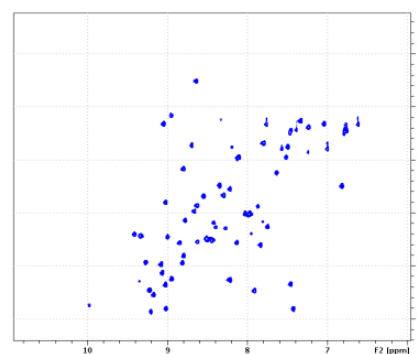
**Number of residues:** 71

**MW (unlabeled):** 8.39 kDa

**Theoretical pI:** 6.64

**UniProt Accession:** P07751

Catalog No.	Label
NEX-SH3-U	unlabeled
NEX-SH3-N	( <sup>15</sup> N, 95%)
NEX-SH3-CN-5	( <sup>13</sup> C, 5%; <sup>15</sup> N, 95%)
NEX-SH3-CN	( <sup>13</sup> C, 95%; <sup>15</sup> N, 95%)
NEX-SH3-CDN	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%)
NEX-SH3-ILV	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%; <sup>13</sup> CH <sub>3</sub> -ILV)
NEX-SH3-ILV FY	( <sup>13</sup> C, 95%; D, 95%; <sup>15</sup> N, 95%; <sup>13</sup> CH <sub>3</sub> -ILV FY)



### Protein Sequence

MGHHHHHHSHMDETGKELVLALYDYQEKSPREVTMKGDLTLNSTNKDWWKVEVNDROF  
VPAAYVKKLD

## Nucleic Acids

Although solution-state NMR is a powerful and versatile tool for studying the structure and dynamics of RNA and DNA, using  $^{13}\text{C}$  correlation spectroscopy to determine structure of RNA in the solid-state<sup>1</sup> shows promise. The different types of information that may be gained from NMR studies of RNA and DNA<sup>2</sup> include base-pairing patterns, conformational equilibria, site-specific information regarding ligand binding, delineation of secondary structure motifs such as hairpins and bulges, local structure and dynamics, and global structure derived from residual dipolar couplings.

The most popular approaches to produce labeled RNA molecules for NMR studies use enzymatic *in vitro* transcription methods that employ labeled rNTPs, T7 RNA polymerase, and either linearized plasmids or double-stranded DNA as templates. These techniques are used to construct labeled RNA molecules of which all of one type of nucleotide is labeled. Perdeuterated NTPs can be used in combination with protonated NTPs to create RNA molecules in which specific types of nucleotides are protonated, thus allowing spectral editing without the significant signal broadening associated with  $^{13}\text{C}$  incorporation.<sup>3</sup>

Because severe signal degeneracy has hampered NMR studies of larger RNAs, key researchers in this area have utilized selectively deuterated rNTPs, in conjunction with *in vitro* synthesis methods, to reduce spectral complexity, spectral line-widths, and for observing NOEs over larger distances.<sup>3</sup>

Labeled DNA oligonucleotides are routinely synthesized using enzymatic *in vitro* methods that utilize labeled dNTPs, a DNA polymerase, and a cDNA template. One advantage of using enzymatic methods over phosphoramidite chemistry is that large oligonucleotides (e.g., >50 nucleotides in length) can be prepared in milligram quantities. Position-specific labeled DNA molecules can be synthesized using standard phosphoramidite chemistry (using CIL's deoxyphosphoramidites) to overcome the limited chemical-shift dispersion of DNA, as well as to create residue-specific probes to obtain functional, structural and dynamic information.

CIL is pleased to offer labeled mono- and triphosphate ribonucleotides, deoxyribonucleotides, and phosphoramidites for use in NMR investigations.

### References

1. Cherepanov, A.V.; Glaubitz, C.; Schwalbe, H. **2010**. High-resolution studies of uniformly  $^{13}\text{C}$ ,  $^{15}\text{N}$ -labeled RNA by solid-state NMR spectroscopy. *Angew Chem Int Ed Engl*, 49(28), 4747-4750.
2. Furtig, B.; Wohnert, C.; Schwalbe, H. **2003**. NMR spectroscopy of RNA. *Chembiochem*, 4(10), 936-962.
3. Lu, K.; Miyazaki, Y.; Summers, M. **2010**. Isotope labeling strategies for NMR studies of RNA. *J Biomol NMR*, 46(1), 113-125.

Cassia LLC was founded in 2005 by noted NMR spectroscopists Drs. Jamie Williamson and Lincoln Scott. CIL and Cassia have a special relationship that combines CIL's isotopic material production and marketing with Cassia's unique expertise in RNA and DNA biosynthesis. Since 2005, CIL and Cassia have developed the most extensive product line of stable isotope-labeled RNA and DNA triphosphates, DNA phosphoramidites and other related compounds. All of these products are routinely available for immediate shipment from stock at CIL.



### Top 10 Reasons to Use Ammonium Salts

1. Self-buffering (pH ~7.6).
2. "Soft cation."
3. Nucleotides of ammonium salts are active with polymerases, synthetases, and phosphatases.
4. Volatile counter-ion.
5. The ammonium cation can be easily exchanged using DOWEX cation exchange resin.
6. The pH does not change during drying of the nucleotide (i.e. "speed-vac," lyophilize).
7. Stoichiometry between the counter-ion and the nucleotide is preserved.
8. Routinely compatible in down-stream syntheses.
9. Compatible in a variety of down-stream chromatography applications.
10. Tested to be comparable in side-by-side *in vitro* transcription reactions!

“We’ve been using CIL’s perdeuterated rNTPs for many years to facilitate NMR studies of larger RNAs. Their newer products, which include partially deuterated rNTPs, have been extraordinarily helpful, and have enabled high resolution NMR structural studies of RNAs that were previously intractable.”

Michael Summers, PhD  
Howard Hughes Medical Institute  
University of Maryland, Baltimore County

## Ribonucleic Acids

### Ribonucleoside Triphosphates ( $^{13}\text{C}$ , $^{15}\text{N}$ , $^{13}\text{C}/^{15}\text{N}$ , D)

Catalog No.	Description
<b>NEW!</b> CLM-8426-CA	Adenosine 5'-triphosphate, ammonium salt ( $^{13}\text{C}_{10}$ , 99%) (in solution) CP 95%
<b>NEW!</b> CLM-10987-CA	Cytidine 5'-triphosphate, ammonium salt ( $^{13}\text{C}_9$ , 99%) (in solution) CP 95%
<b>NEW!</b> CLM-10988-CA	Guanosine 5'-triphosphate, ammonium salt ( $^{13}\text{C}_{10}$ , 99%) (in solution) CP 90%
<b>NEW!</b> CLM-10914-CA	Uridine 5'-triphosphate, ammonium salt ( $^{13}\text{C}_9$ , 99%) (in solution) CP 90%
NLM-3987-CA	Adenosine 5'-triphosphate, ammonium salt ( $^{15}\text{N}_5$ , 98-99%) (in solution) CP 90%
NLM-4266-CA	Cytidine 5'-triphosphate, ammonium salt ( $^{15}\text{N}_3$ , 96%) (in solution) CP 90%
NLM-4268-CA	Guanosine 5'-triphosphate, ammonium salt ( $^{15}\text{N}_5$ , 98-99%) (in solution) CP 90%
NLM-4270-CA	Uridine 5'-triphosphate, ammonium salt ( $^{15}\text{N}_3$ , 98-99%) (in solution) CP 90%
NLM-7519-CA	Set of 4 Ribonucleoside 5'-triphosphates ( $^{15}\text{N}$ , 98%) (ammonium salt/in solution) CP 90%
CNLM-4265-CA	Adenosine 5'-triphosphate, ammonium salt ( $^{13}\text{C}$ , $^{15}\text{N}$ ; 98-99%) (in solution) CP 90%
CNLM-4267-CA	Cytidine 5'-triphosphate, ammonium salt ( $^{13}\text{C}$ , $^{15}\text{N}$ ; 96-98%) (in solution) CP 90%
CNLM-4269-CA	Guanosine 5'-triphosphate, ammonium salt ( $^{13}\text{C}$ , $^{15}\text{N}$ ; 98-99%) (in solution) CP 90%
CNLM-4271-CA	Uridine 5'-triphosphate, ammonium salt ( $^{13}\text{C}$ , $^{15}\text{N}$ ; 98-99%) (in solution) CP 90%
CNLM-7503-CA	Set of 4 Ribonucleoside 5'-triphosphates ( $^{13}\text{C}$ , $^{15}\text{N}$ ; 98-99%) (ammonium salt/in solution) CP 90%
DLM-7514-CA	Adenosine 5'-triphosphate, ammonium salt ( $\text{D}_8$ , 97%) (in solution) CP 90%
DLM-7515-CA	Cytidine 5'-triphosphate, ammonium salt ( $\text{D}_8$ , 97%) (in solution) CP 90%
DLM-7516-CA	Guanosine 5'-triphosphate, ammonium salt ( $\text{D}_7$ , 97%) (in solution) CP 90%
DLM-7517-CA	Uridine 5'-triphosphate, ammonium salt ( $\text{D}_8$ , 97%) (in solution) CP 90%
DLM-7518-CA	Set of 4 Ribonucleoside 5'-triphosphates (D, 98%) (ammonium salt/in solution) CP 90%

Please inquire for lithium salts.

#### References

- Song, Y.; Marmion, R.A.; Park, J.O.; et al. **2017**. Dynamic control of dNTP synthesis in early embryos. *Dev Cell*, 42(3), 301-308.
- Le, M.T.; Brown, R.E.; Simon, A.E.; et al. **2015**. *In vivo*, large-scale preparation of uniformly N-15 and site-specifically C-13 labeled homogeneous, recombinant RNA for NMR studies. *Methods Enzymol*, 565, 495-535.
- Alverado, L.J.; Longhini, A.P.; LeBlanc, R.M.; et al. **2014**. Chemo-enzymatic synthesis of selectively  $^{13}\text{C}/^{15}\text{N}$  labeled RNA for NMR structural and dynamics studies. *Methods Enzymol*, 549, 133-162.
- Lu, K.; Miyazaki, Y.; Summers, M.F. **2010**. Isotope labeling strategies for NMR studies of RNAs. *J Biomol NMR*, 46(1), 113-125.
- Dayie, K.T. **2008**. Key labeling technologies to tackle sizeable problems in RNA structural biology. *Int J Mol Sci*, 9(7), 1214-1240.
- Nikonowicz, E.P.; Sirr, A.; Legault, P.; et al. **1992**. Preparation of C-13 and N-15 labeled RNAs for heteronuclear multidimensional NMR studies. *Nucleic Acids Res*, 20(17), 4507-4513.

## Fluorine-Labeled Nucleotide 5'-Triphosphates

**New!**

The fluorine nucleus is extremely sensitive to the local chemical environment, which leads to a wide chemical shift range. This makes it an excellent probe for secondary structure, especially where the chemical shift dispersion is limited, such as in RNA. Although researchers have used  $^{19}\text{F}$ -NMR to study nucleic acids for decades, only recently has a larger RNA been uniformly labeled with fluorine and investigated using  $^{19}\text{F}$ -NMR.<sup>1</sup>

CIL is pleased to offer new fluorinated nucleotide 5'-triphosphates for use in studying structure and dynamics of RNA using  $^{19}\text{F}$ -NMR.

#### References

1. Sochor, F.; Silvers, R.; Muller, D.; et al. **2016**.  $^{19}\text{F}$ -labeling of the adenine H2-site to study large RNAs by NMR spectroscopy. *J Biomol NMR*, 64(1), 63-74.

Catalog No.	Description
ULM-10696-CA	5-Fluorouridine 5'-triphosphate (5F-UTP)
ULM-10697-CA	5-Fluorocytidine 5'-triphosphate (5F-CTP)
ULM-10698-CA	2-Fluoroadenosine 5'-triphosphate (2F-ATP)

“When my lab makes labeled RNA, we count on high yields and purity. We have been using  $^{13}\text{C}/^{15}\text{N}$  ribonucleotide triphosphates (rNTPs) and selectively deuterated rNTPs from CIL for the past 15 years. We have always been very happy with the performance and quality of the CIL rNTPs. They are the gold standard.”

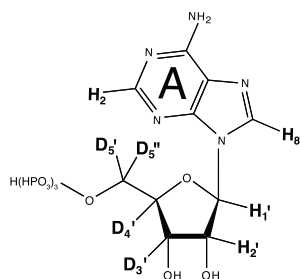
Samuel Butcher, PhD  
Department of Biochemistry  
University of Wisconsin-Madison

Chemical purity (CP) is 98% or greater, unless otherwise specified.  
For research use only. Not for use in diagnostic procedures.

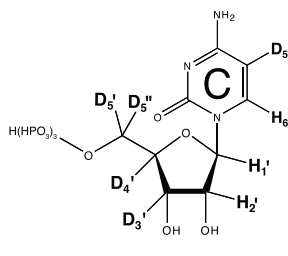
## Ribonucleic Acids

### Ribonucleoside Triphosphates (Selectively Labeled D)

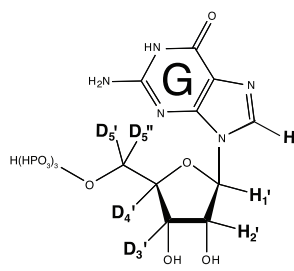
Catalog No.	Description
DLM-7862	Equimolar Mix: ATP, GTP, (ribose-3',4',5',5''-D <sub>4</sub> , 98%); CTP, UTP (5-D, ribose-3',4',5',5''-D <sub>4</sub> , 98%) NH <sub>4</sub> <sup>+</sup> salt (see structures below)
DLM-8815-CA	Adenosine 5'-triphosphate, ammonium salt (2-D, 97%) (in solution) CP 90%
DLM-9619-CA	Adenosine 5'-triphosphate, ammonium salt (ribose-1',2',3',4',5',5''-D <sub>6</sub> , 98%) (in solution) CP 90%
DLM-9366-CA	Cytidine 5'-triphosphate, ammonium salt (cytosine-5-D, 97%) (in solution) CP 90%
DLM-9267-CA	Cytidine 5'-triphosphate, ammonium salt (5,6-D <sub>2</sub> , 97%) (in solution) CP 90%
DLM-8594-CA	Cytidine 5'-triphosphate, ammonium salt (cytosine-5-D, 6-H; ribose-1,2,3,4,5,5-D <sub>6</sub> , 96-97%) (in solution)
DLM-9365-CA	Uridine 5'-triphosphate, ammonium salt (uracil-5-D, 98%) (in solution) CP 90%
DLM-9100-CA	Uridine 5'-triphosphate, ammonium salt (5,6-D <sub>2</sub> , 98%) (in solution) CP 90%
DLM-8637-CA	Uridine 5'-triphosphate, ammonium salt (uracil-5-D, 6-H; ribose-1,2,3,4,5,5-D <sub>6</sub> , 96-97%) (in solution)



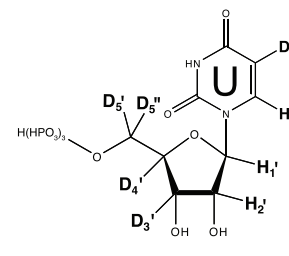
DLM-8922



DLM-8924

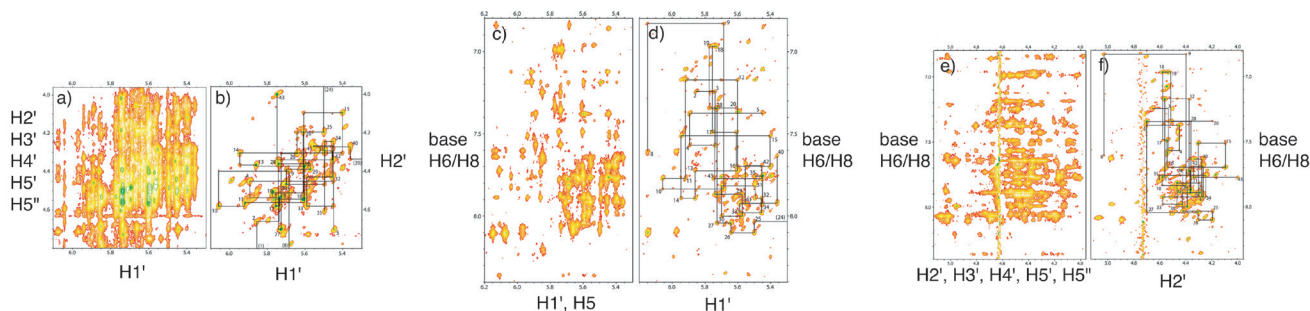


DLM-8923



DLM-8925

DLM-8922-CA	Adenosine-5'-triphosphate, ammonium salt (ribose-3',4',5',5''-D <sub>4</sub> , 98%) (in solution)
DLM-8924-CA	Cytidine-5'-triphosphate, ammonium salt (5-D, ribose-3',4',5',5''-D <sub>4</sub> , 98%) (in solution)
DLM-8923-CA	Guanosine-5'-triphosphate, ammonium salt (ribose-3',4',5',5''-D <sub>4</sub> , 98%) (in solution)
DLM-8925-CA	Uridine-5'-triphosphate, ammonium salt (5-D, ribose-3',4',5',5''-D <sub>4</sub> , 98%) (in solution)



<sup>1</sup>H-<sup>1</sup>H-NOESY Spectra of the Tetraloop-Receptor RNA (45 nt dimer; 30 kDa). Spectra above are from unlabeled RNA (a, c, e) and selectively deuterated RNA (b, d, f). The selectively deuterated RNA was prepared using the equimolar mix (CIL catalog no. DLM-7862). The left two panels (a, b) contain NOEs between the H1' proton and all other ribose protons. The middle two panels (c, d) contain NOEs between the base protons and H1' protons. The right two panels (e, f) contain NOEs between the base and other ribose protons. Spectra taken from Davis, J.H., et al. (see reference below) were provided courtesy of professor Sam Butcher at the University of Wisconsin. The sequential assignment pattern of inter- and intranucleotide NOEs is shown for the D<sub>5</sub>-RNA. The advantages of the selectively deuterated pattern are evident in these key regions of the spectra.

#### Reference

Davis, J.H.; Tonelli, M.; Scott, L.G.; et al. **2005**. Helical packing in solution: NMR structure of a 30 kDa GAAA tetraloop-receptor complex. *J Mol Biol*, 351(2), 371-382.

Chemical purity (CP) is 98% or greater, unless otherwise specified.  
For research use only. Not for use in diagnostic procedures.



## Ribonucleic Acids

### Ribonucleoside Monophosphates ( $^{15}\text{N}$ , $^{13}\text{C}/^{15}\text{N}$ )

Catalog No.	Description
NLM-3792	Adenosine 5'-monophosphate, lithium salt ( $^{15}\text{N}_5$ , 96-98%) (in solution)
NLM-3793	Cytidine 5'-monophosphate, lithium salt ( $^{15}\text{N}_3$ , 96-98%) (in solution)
NLM-3794	Guanosine 5'-monophosphate, lithium salt ( $^{15}\text{N}_5$ , 96-98%) (in solution)
NLM-3795	Uridine 5'-monophosphate, lithium salt ( $^{15}\text{N}_2$ , 96-98%) (in solution)
CNLM-3802	Adenosine 5'-monophosphate, lithium salt ( $^{13}\text{C}_{10}$ , 98%; $^{15}\text{N}_5$ , 96-98%) (in solution)
CNLM-3803	Cytidine 5'-monophosphate, lithium salt ( $^{13}\text{C}_9$ , 98%; $^{15}\text{N}_3$ , 96-98%) (in solution)
CNLM-3804	Guanosine 5'-monophosphate, lithium salt ( $^{13}\text{C}_{10}$ , 98%; $^{15}\text{N}_5$ , 96-98%) (in solution)
CNLM-3805	Uridine 5'-monophosphate, lithium salt ( $^{13}\text{C}_9$ , 98%; $^{15}\text{N}_2$ , 96-98%) (in solution) CP 90%

## Deoxyribonucleic Acids

### Deoxyribonucleoside Triphosphates ( $^{15}\text{N}$ , $^{13}\text{C}/^{15}\text{N}$ , D)

NLM-6217-CA	2'-Deoxyguanosine 5'-triphosphate, ammonium salt ( $^{15}\text{N}_5$ , 98-99%) (in solution) CP 90%
NLM-6215	2'-Deoxyadenosine 5'-triphosphate, lithium salt ( $^{15}\text{N}_5$ , 98%) (in solution) CP 90%
NLM-6216	2'-Deoxycytidine 5'-triphosphate, lithium salt ( $^{15}\text{N}_3$ , 98%) (in solution) CP 90%
NLM-6217	2'-Deoxyguanosine 5'-triphosphate, lithium salt ( $^{15}\text{N}_5$ , 98%) (in solution) CP 90%
NLM-6218	Thymidine 5'-triphosphate, lithium salt ( $^{15}\text{N}_2$ , 98%) (in solution) CP 90%
NLM-7512	Set of 4 2'-Deoxyribonucleoside 5'-triphosphates ( $^{15}\text{N}$ , 98%) (lithium salt/in solution) CP 90%
CNLM-6219-CA	2'-Deoxyadenosine 5'-triphosphate, ammonium salt ( $^{13}\text{C}_{10}$ , 98%; $^{15}\text{N}_5$ , 97-98%) CP 90%
CNLM-6221-CA	2'-Deoxyguanosine 5'-triphosphate, ammonium salt ( $^{13}\text{C}_{10}$ , 98%; $^{15}\text{N}_5$ , 96-98%) CP 90%
CNLM-6219	2'-Deoxyadenosine 5'-triphosphate, lithium salt ( $^{13}\text{C}_{10}$ , 98%; $^{15}\text{N}_5$ , 98%) (in solution) CP 90%
CNLM-6220	2'-Deoxycytidine 5'-triphosphate, lithium salt ( $^{13}\text{C}_9$ , 98%; $^{15}\text{N}_3$ , 98%) (in solution) CP 90%
CNLM-6221	2'-Deoxyguanosine 5'-triphosphate, lithium salt ( $^{13}\text{C}_{10}$ , 98%; $^{15}\text{N}_5$ , 98%) (in solution) CP 90%
CNLM-6222	Thymidine 5'-triphosphate, lithium salt ( $^{13}\text{C}_{10}$ , 98%; $^{15}\text{N}_2$ , 98%) (in solution) CP 90%
CNLM-7513	Set of 4 2'-Deoxyribonucleoside 5'-triphosphates ( $^{13}\text{C}$ , 98%; $^{15}\text{N}$ , 98%) (lithium salt/in solution) CP 90%
DLM-7507	2-Deoxyadenosine 5'-triphosphate, lithium salt (D, 97%) (in solution) CP 90%
DLM-7508	2-Deoxycytidine 5'-triphosphate, lithium salt (D, 97%) (in solution) CP 90%
DLM-7509	2-Deoxyguanosine 5'-triphosphate, lithium salt (D, 97%) (in solution) CP 90%
DLM-7510	Thymidine 5'-triphosphate, lithium salt (D, 97%) (in solution) CP 90%
DLM-7511	Set of 4 2'-Deoxyribonucleoside 5'-triphosphates (D, 98%) (lithium salt/in solution) CP 90%

### Deoxyribonucleoside Monophosphates ( $^{15}\text{N}$ , $^{13}\text{C}/^{15}\text{N}$ )

NLM-3919	2'-Deoxyadenosine 5'-monophosphate, lithium salt ( $^{15}\text{N}_5$ , 98%) (in solution)
NLM-3921	2'-Deoxycytidine 5'-monophosphate, lithium salt ( $^{15}\text{N}_3$ , 96%) (in solution)
NLM-6835	2'-Deoxyguanosine 5'-monophosphate, lithium salt ( $^{15}\text{N}$ , 98%) (in solution)
NLM-3925	Thymidine 5'-monophosphate, lithium salt ( $^{15}\text{N}_2$ , 98%) (in solution)
CNLM-3918	2'-Deoxyadenosine 5'-monophosphate, lithium salt ( $^{13}\text{C}_{10}$ , 98%; $^{15}\text{N}_5$ , 98%) (in solution)
CNLM-6834	2'-Deoxycytidine 5'-monophosphate, lithium salt ( $^{13}\text{C}$ , 98%; $^{15}\text{N}$ , 98%) (in solution)
CNLM-6836	2'-Deoxyguanosine 5'-monophosphate, lithium salt ( $^{13}\text{C}$ , 98%; $^{15}\text{N}$ , 98%) (in solution)
CNLM-3924	Thymidine 5'-monophosphate, lithium salt ( $^{13}\text{C}_{10}$ , 98%; $^{15}\text{N}_2$ , 98%) (in solution)
CNLM-7871	Set of 4 2'-Deoxyribonucleoside 5'-monophosphates ( $^{13}\text{C}$ , 98%; $^{15}\text{N}$ , 98%) (lithium salt/in solution)

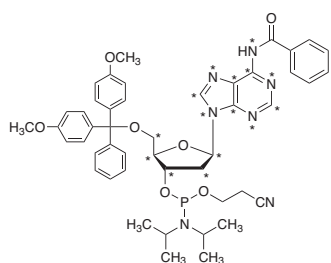
“Without these labeled rNTPs from CIL we would not have been able to prepare such high-quality samples which made the assignment and structure determination possible.”

Michael Durney, PhD  
Department of Molecular and Cellular Biology  
Harvard University

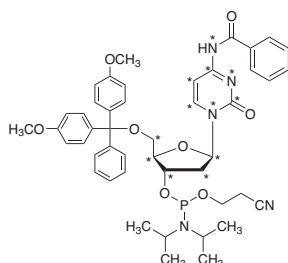
## Deoxyribonucleic Acids

### Deoxyribophosphoramidites ( $^{13}\text{C}/^{15}\text{N}$ )

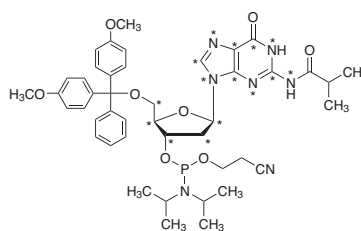
Catalog No.	Description
CNLM-6828	2'-Deoxyadenosine phosphoramidite ( $^{13}\text{C}_{10}$ , 98%; $^{15}\text{N}_5$ , 98%) CP 95%
CNLM-6830	2'-Deoxycytidine phosphoramidite ( $^{13}\text{C}_9$ , 98%; $^{15}\text{N}_3$ , 98%) CP 95%
CNLM-6825	2'-Deoxyguanosine phosphoramidite ( $^{13}\text{C}_{10}$ , 98%; $^{15}\text{N}_5$ , 98%) CP 95%
CNLM-6824	Thymidine phosphoramidite ( $^{13}\text{C}_{10}$ , 98%; $^{15}\text{N}_2$ , 98%) CP 95%



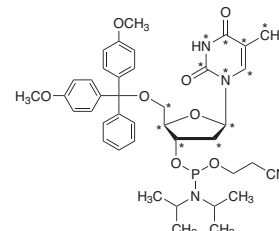
CNLM-6828



CNLM-6830



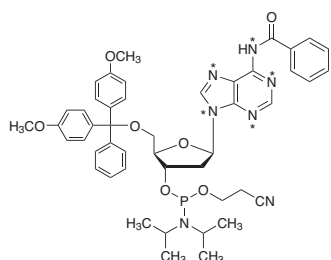
CNLM-6825



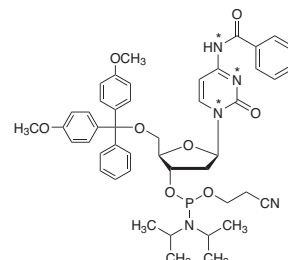
CNLM-6824

### Deoxyribophosphoramidites ( $^{15}\text{N}$ )

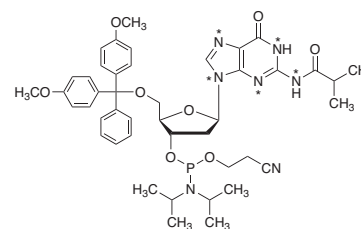
NLM-6829	2'-Deoxyadenosine phosphoramidite ( $^{15}\text{N}_5$ , 98%) CP 95%
NLM-6827	2'-Deoxycytidine phosphoramidite ( $^{15}\text{N}_3$ , 98%) CP 95%
NLM-6826	2'-Deoxyguanosine phosphoramidite ( $^{15}\text{N}_5$ , 98%) CP 95%
NLM-6823	Thymidine phosphoramidite ( $^{15}\text{N}_2$ , 96-98%) CP 95%



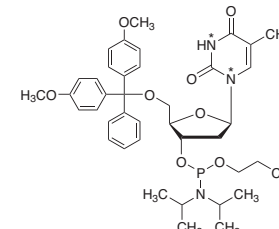
NLM-6829



NLM-6827



NLM-6826



NLM-6823

“CIL has been a strong supporter of NMR methods of development over the years, providing critical isotope-enriched reagents for research and development, without which many of the recent advances in biomolecular NMR would simply not have been possible. In particular, the broad biological impact and tremendous success of the multi-dimensional triple-resonance biomolecular NMR would not have been achieved without the high quality and broadly accessible reagents that CIL has provided to the scientific community over the last 35 years.”

Gaetano Montelione, PhD  
Distinguished Professor of Molecular Biology  
and Biochemistry, Rutgers University  
Founder, Nexomics Biosciences

## Miscellaneous RNA and DNA Products

Catalog No.	Description
CLM-1654	Adenine (8- <sup>13</sup> C, 95%)
NLM-6924	Adenine-HCl ( <sup>15</sup> N <sub>5</sub> , 98%)
CLM-3698	Adenosine (ribose-2- <sup>13</sup> C, 99%)
CLM-7674	Adenosine (3'- <sup>13</sup> C, 98%)
CLM-3678	Adenosine (ribose- <sup>13</sup> C <sub>5</sub> , 98%) CP 97%
DLM-7676	Adenosine (ribose-1-D, 98%)
DLM-7677	Adenosine (ribose-2-D, 97%)
DLM-7678	Adenosine (ribose-5,5-D <sub>2</sub> , 98%)
NLM-9750	Adenosine ( <sup>15</sup> N <sub>5</sub> , 96-98%)
CNLM-3806-CA	Adenosine ( <sup>13</sup> C <sub>10</sub> , 98%; <sup>15</sup> N <sub>5</sub> , 96-98%)
CLM-3605	Adenosine-H <sub>2</sub> O (ribose-1- <sup>13</sup> C, 99%) CP 95%
CLM-3611	Cytidine (ribose-1- <sup>13</sup> C, 99%)
CLM-3699	Cytidine (ribose-2- <sup>13</sup> C, 99%)
CLM-3679	Cytidine (ribose- <sup>13</sup> C <sub>5</sub> , 98%)
DLM-7681	Cytidine (ribose-5,5-D <sub>2</sub> , 98%)
NLM-3797	Cytidine ( <sup>15</sup> N <sub>3</sub> , 96-98%)
CNLM-3807	Cytidine ( <sup>13</sup> C <sub>9</sub> , 98%; <sup>15</sup> N <sub>3</sub> , 96-98%)
CNLM-4424	Cytosine (2- <sup>13</sup> C, 99%; 1,3- <sup>15</sup> N <sub>2</sub> , 98%)
NLM-3895	2'-Deoxyadenosine ( <sup>15</sup> N <sub>5</sub> , 96-98%)
CLM-3700	2'-Deoxyadenosine-H <sub>2</sub> O (deoxyribose-1- <sup>13</sup> C, 99%)
CLM-3701	2'-Deoxyadenosine-H <sub>2</sub> O (deoxyribose-2- <sup>13</sup> C, 99%)
CLM-7682	2'-Deoxyadenosine-H <sub>2</sub> O (ribose-5- <sup>13</sup> C, 98%)
CLM-4579	2'-Deoxyadenosine-H <sub>2</sub> O (ribose- <sup>13</sup> C <sub>5</sub> , 99%)
DLM-7683	2'-Deoxyadenosine-H <sub>2</sub> O (ribose-5,5-D <sub>2</sub> , 98%)
CNLM-3896-CA	2'-Deoxyadenosine-H <sub>2</sub> O ( <sup>13</sup> C <sub>10</sub> , 98%; <sup>15</sup> N <sub>5</sub> , 96-98%)
NLM-3897	2'-Deoxycytidine ( <sup>15</sup> N <sub>3</sub> , 96-98%)
CNLM-3898	2'-Deoxycytidine ( <sup>13</sup> C <sub>9</sub> , 98%; <sup>15</sup> N <sub>3</sub> , 96-98%)
CLM-7684	2'-Deoxycytidine-H <sub>2</sub> O (ribose-1- <sup>13</sup> C, 98%)
CLM-3702	2'-Deoxycytidine-H <sub>2</sub> O (deoxyribose-2- <sup>13</sup> C, 99%)
DLM-7685	2'-Deoxycytidine-H <sub>2</sub> O (ribose-5,5-D <sub>2</sub> , 98%)
DLM-6142	5-Methyl-2'-deoxycytidine-HCl (methyl-D <sub>3</sub> , ring-6-D, 96-98%) CP 95%
CLM-7686	2'-Deoxyguanosine-H <sub>2</sub> O (ribose-1- <sup>13</sup> C, 98%)
DLM-7687	2'-Deoxyguanosine-H <sub>2</sub> O (ribose-5,5-D <sub>2</sub> , 98%)
NLM-3899-CA	2'-Deoxyguanosine-H <sub>2</sub> O ( <sup>15</sup> N <sub>5</sub> , 98%) CP 95%
CNLM-3900-CA	2'-Deoxyguanosine-H <sub>2</sub> O ( <sup>13</sup> C <sub>10</sub> , 98%; <sup>15</sup> N <sub>5</sub> , 96-98%)
NLM-6715	8-Hydroxy-2'-deoxyguanosine ( <sup>15</sup> N <sub>5</sub> , 98%) CP 95%
CNLM-8771-CA	2'-Deoxyuridine, ammonium salt ( <sup>13</sup> C <sub>9</sub> , 98-99%; <sup>15</sup> N <sub>2</sub> , 98-99%) (in solution) CP 90%
CNLM-10422	2'-Fluoro-2'-deoxyuridine (uracil- <sup>13</sup> C <sub>4</sub> , 99%; <sup>15</sup> N <sub>2</sub> , 98%)
DLM-4391	5,6-Dihydrothymine (5,6,6-D <sub>3</sub> , methyl-D <sub>3</sub> , 95%)
CNLM-4510	5,6-Dihydrouracil ( <sup>13</sup> C <sub>4</sub> , 99%; <sup>15</sup> N <sub>2</sub> , 98%)

Catalog No.	Description
NLM-798	5-Fluorouracil (1,3- <sup>15</sup> N <sub>2</sub> , 99%)
CNLM-3916	5-Fluorouracil ( <sup>13</sup> C <sub>4</sub> , 99%; <sup>15</sup> N <sub>2</sub> , 98%)
CLM-1019	Guanine (8- <sup>13</sup> C, 98%)
NLM-6925	Guanine ( <sup>15</sup> N <sub>5</sub> , 98%)
CNLM-3990	Guanine (8- <sup>13</sup> C, 98%; 7,9- <sup>15</sup> N <sub>2</sub> , 98%)
NLM-3798	Guanosine ( <sup>15</sup> N <sub>5</sub> , 96-98%)
CLM-7688	Guanosine-H <sub>2</sub> O (ribose-1- <sup>13</sup> C, 98%)
CNLM-3808-CA	Guanosine-H <sub>2</sub> O ( <sup>13</sup> C <sub>10</sub> , 98%; <sup>15</sup> N <sub>5</sub> , 96-98%)
DLM-7689	Guanosine-H <sub>2</sub> O (ribose-5,5-D <sub>2</sub> , 98%)
CNLM-3832	8-Hydroxyadenine (8- <sup>13</sup> C, 98%; 6,9-diamino- <sup>15</sup> N <sub>2</sub> , 98%)
CNLM-4392	5-Hydroxycytosine (2- <sup>13</sup> C, 99%; 1,3- <sup>15</sup> N <sub>2</sub> , 98%)
DLM-7471	3-Methyladenine (methyl-D <sub>3</sub> , 98%)
DLM-7473	6-O-Methylguanine (methyl-D <sub>3</sub> , 98%)
DLM-7472	7-Methylguanine (methyl-D <sub>3</sub> , 98%)
CNLM-10505	2-Pyrimidinone (2- <sup>13</sup> C, 99%; <sup>15</sup> N <sub>2</sub> , 98%)
CLM-3629	Ribothymidine (ribose-1- <sup>13</sup> C, 99%)
NLM-10691	α-Thymidine ( <sup>15</sup> N <sub>2</sub> , 98%)
CLM-3647	Thymidine (methyl- <sup>13</sup> C, 98%)
CLM-4289	Thymidine (deoxyribose-1- <sup>13</sup> C, 99%)
CLM-3703	Thymidine (deoxyribose-2- <sup>13</sup> C, 99%)
CLM-7692	Thymidine (deoxyribose-3- <sup>13</sup> C, 99%)
DLM-7691	Thymidine (deoxyribose-5,5-D <sub>2</sub> , 98%)
NLM-3901	Thymidine ( <sup>15</sup> N <sub>2</sub> , 96-98%) CP 97%
CNLM-3902	Thymidine ( <sup>13</sup> C <sub>10</sub> , 98%; <sup>15</sup> N <sub>2</sub> , 96-98%)
DLM-1089	Thymine (α,α,α,6-D <sub>4</sub> , 98%)
NLM-3995	Thymine (1,3- <sup>15</sup> N <sub>2</sub> , 98%)
CNLM-6945	Thymine ( <sup>13</sup> C <sub>5</sub> , 98%; <sup>15</sup> N <sub>2</sub> , 98%)
CLM-3276	Uracil (2- <sup>13</sup> C, 99%)
CLM-692	Uracil (4,5- <sup>13</sup> C <sub>2</sub> , 99%)
CLM-10507	Uracil ( <sup>13</sup> C <sub>4</sub> , 99%)
DLM-8633	Uracil (5-D, 98%)
DLM-8502	Uracil (5,6-D <sub>2</sub> , 98%)
NLM-637	Uracil (1,3- <sup>15</sup> N <sub>2</sub> , 98%)
CNLM-3917	Uracil ( <sup>13</sup> C <sub>4</sub> , 99%; <sup>15</sup> N <sub>2</sub> , 98%)
CLM-3630	Uridine (ribose-1- <sup>13</sup> C, 99%)
CLM-3680	Uridine (ribose- <sup>13</sup> C <sub>5</sub> , 98%)
DLM-7693	Uridine (ribose-5,5-D <sub>2</sub> , 98%)
NLM-812	Uridine ( <sup>15</sup> N <sub>2</sub> , 98%)
CNLM-3809	Uridine ( <sup>13</sup> C <sub>9</sub> , 98%; <sup>15</sup> N <sub>2</sub> , 96-98%)

Chemical purity (CP) is 98% or greater, unless otherwise specified.  
For research use only. Not for use in diagnostic procedures.

## Deuterated Buffers

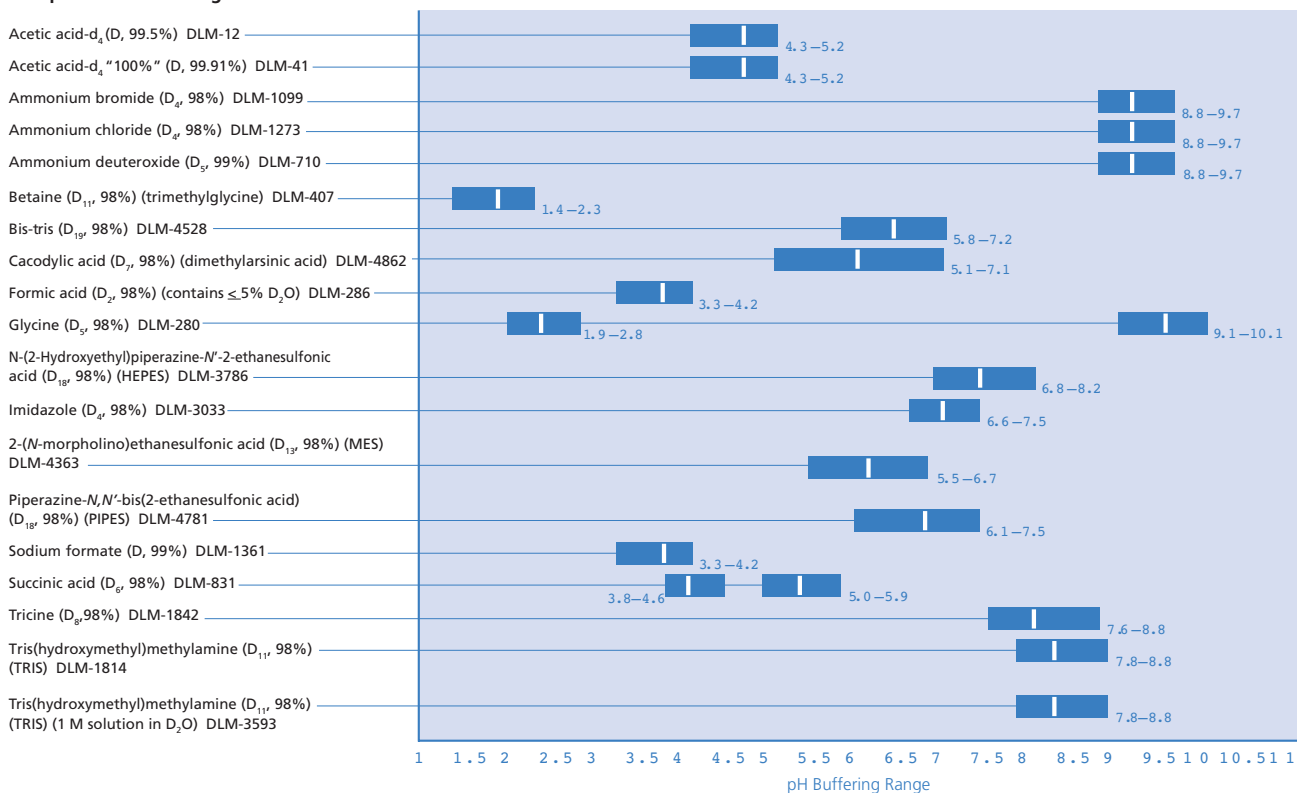
Catalog No.	Description
DLM-12	Acetic acid- $D_4$ ( $D$ , 99.5%)
DLM-41	Acetic acid- $d_4$ "100%" ( $D$ , 99.91%)
DLM-1099	Ammonium bromide ( $D_4$ , 98%)
DLM-1273	Ammonium chloride ( $D_4$ , 98%)
DLM-710	Ammonium deuterioxide ( $D_5$ , 99%) ~25% in solution $D_2O$
DLM-407	Betaine ( $D_{11}$ , 98%)
DLM-4528	Bis-tris ( $D_{19}$ , 98%)
DLM-4862	Cacodylic acid ( $D_7$ , 98%)
DLM-286	Formic acid ( $D_2$ , 98%) <5% $D_2O$
DLM-280	Glycine ( $D_5$ , 98%)

Catalog No.	Description
DLM-3786	HEPES ( $D_{18}$ , 98%)
DLM-3033	Imidazole ( $D_4$ , 98%)
DLM-4363	MES ( $D_{13}$ , 98%)
DLM-4781	PIPES ( $D_{18}$ , 98%)
DLM-1361	Sodium formate ( $D$ , 98%)
DLM-831	Succinic acid ( $D_6$ , 98%)
DLM-1842	Tricine ( $D_8$ , 98%)
DLM-4779	Trimethylamine <i>N</i> -oxide ( $D_9$ , 98%)
DLM-1814	TRIS ( $D_{11}$ , 98%)
DLM-3593	TRIS ( $D_{11}$ , 98%) 1 M in $D_2O$

## pH Buffering Range Chart

### Compound and Catalog Number

$pK_a$  is indicated by a white rule within the range



## Solvent Data Chart

	<sup>1</sup> H Chemical shift (ppm from TMS) (multiplicity) ✱	JHD (Hz)	<sup>13</sup> C Chemical shift (ppm from TMS) (multiplicity) ✱	JCD (Hz)	<sup>1</sup> H Chemical shift of HOD (ppm from TMS) ●	Density at 20°C □	Melting point (°C) □	Boiling point (°C) □	Dielectric constant (unlabeled)	Molecular weight □
Acetic acid-d <sub>4</sub>	11.65 (1) 2.04 (5)	2.2	178.99 (1) 20.0 (7)	20.0	11.5*	1.12	16.7	118	6.1	64.08
Acetone-d <sub>6</sub>	2.05 (5)	2.2	206.68 (1) 29.92 (7)	00.9 19.4	2.8*	0.87	-94	56.5	20.7	64.12
Acetonitrile-d <sub>3</sub>	1.94 (5)	2.5	118.69 (1) 1.39 (7)	21.0	2.1*	0.84	-45	81.6	37.5	44.07
Benzene-d <sub>6</sub>	7.16 (1)		128.39 (3)	24.3	0.4*	0.95	5.5	80.1	2.3	84.15
Chloroform-d	7.24 (1)		77.23 (3)	32.0	1.5*	1.50	-63.5	61-62	4.8	120.38
Cyclohexane-d <sub>12</sub>	1.38 (1)		26.43 (5)	19.0	0.8*	0.89	6.47	80.7	2.0	96.24
Deuterium oxide	4.80 (DSS) 4.81 (TSP)		NA	NA	4.8*	1.11	3.81	101.42	78.5	20.03
<i>N,N</i> -Dimethyl-formamide-d <sub>7</sub>	8.03 (1) 2.92 (5) 2.75 (5)	1.9 1.9	163.15 (3) 34.89 (7) 29.76 (7)	29.4 21.0 21.1	3.5*	1.03	-61	153	36.7	80.14
Dimethyl sulfoxide-d <sub>6</sub>	2.50 (5)	1.9	39.51 (7)	21.0	3.3*	1.19	18.55	189	46.7	84.17
1,4-Dioxane-d <sub>8</sub>	3.53 (m)		66.66 (5)	21.9	2.4*	1.13	11.8	101.1	2.2	96.16
Ethanol-d <sub>6</sub>	5.19 (1) 3.56 (1) 1.11 (m)		56.96 (5) 17.31 (7)	22.0 19.0	5.3*	0.89	-114.1	78.5	24.5	52.11
Methanol-d <sub>4</sub>	4.87 (1) 3.31 (5)	1.7	49.15 (7)	21.4	4.9*	0.89	-97.8	64.7	32.7	36.07
Methylene chloride-d <sub>2</sub>	5.32 (3)	1.1	54.00 (5)	27.2	1.5*	1.35	-95	39.75	8.9	86.95
Pyridine-d <sub>5</sub>	8.74 (1) 7.58 (1) 7.22 (1)		150.35 (3) 135.91 (3) 123.87 (3)	27.5 24.5 25.0	5.0*	1.05	-41.6	115.2-115.3	12.4	84.13
1,1,2,2-Tetrachloroethane-d <sub>2</sub>	6.0		73.78 (3)			1.62	-44	146.5	8.20	169.86
Tetrahydrofuran-d <sub>8</sub>	3.58 (1) 1.73 (1)		67.57 (5) 25.37 (5)	22.2 20.2	2.4-2.5	0.99	-108.5	66	7.6	80.16
Toluene-d <sub>8</sub>	7.09 (m) 7.00 (1) 6.98 (5) 2.09 (5)	2.3	137.86 (1) 129.24 (3) 128.33 (3) 125.49 (3) 20.4 (7)	23.0 24.0 24.0 19.0	0.4*	0.94	-95	110.6	2.4	100.19
Trifluoroacetic Acid-d	11.50 (1)		164.2 (4) 116.6 (4)		11.5	1.49	-15.4	72.4		115.03
Trifluoroethanol-d <sub>3</sub>	5.02 (1) 3.88 (4x3)	2(9)	126.3 (4) 61.5 (4x5)	22.0	5.0*	1.41	-43.5	74.05		103.06

M.J. O'Neil, P.E. Heckelman, C.B. Koch, K.J. Roman, *The Merck Index*, an Encyclopedia of Chemicals, Drugs, and Biologicals – Fourteenth Edition, Merck Co., Inc. Whitehouse Station, NJ 2006.

✱ The <sup>1</sup>H spectra of the residual protons and <sup>13</sup>C spectra were obtained on a Varian Gemini 200 spectrometer at 295°K. The NMR solvents used to acquire these spectra contain a maximum of 0.05% and 1.0% TMS (v/v) respectively. Since deuterium has a spin of 1, triplets arising from coupling to deuterium have the intensity ratio of 1:1:1. "m" denotes a broad peak with some fine structures. It should be noted that chemical shifts can be dependent on solvent, concentration and temperature.

● Approximate values only; may vary with pH, concentration, and temperature.  
 □ Melting and boiling points are those of the corresponding unlabeled solvent (except for D<sub>2</sub>O). These temperature limits can be used as a guide to determine the useful liquid range of the solvents. Information gathered from the Merck Index – Fourteenth Edition.

\* HOD Peaks – NMR spectra of "neat" deuterated solvent always exhibit a peak due to H<sub>2</sub>O in addition to the residual solvent peak. When the exchange rate between H<sub>2</sub>O and HOD is slow on the NMR timescale the water peak appears as two peaks, a singlet corresponding to H<sub>2</sub>O and a 1:1:1 triplet corresponding to HOD.

## Application Notes Summaries



### Application Note 15

#### Top Ten Tips for Producing $^{13}\text{C}$ , $^{15}\text{N}$ Protein in Abundance

Deborah A. Berthold, Victoria J. Jeisy, Terry L. Sasser, John J. Shea, Heather L. Frericks, Gautam Shah, and Chad M. Rienstra

What could be easier than overexpressing an *E. coli* protein in *E. coli*? You don't have to be an old hand at protein expression to know that this can often be more difficult than it sounds. We tested our skills recently with DsbA, a 20 kDa protein that catalyzes disulfide bond formation in the *E. coli* periplasm. The wildtype DsbA expressed well in LB medium and also in a BioExpress®-supplemented  $^{13}\text{C}$ ,  $^{15}\text{N}$ -labeling medium. Likewise, the DsbA C33S mutant expressed well in LB. But when we first tried to label C33S, our luck ran out – we saw no expression at all. Today we are producing  $^{13}\text{C}$ ,  $^{15}\text{N}$  DsbA C33S at a yield of 100 mg per liter. Here are our top ten tips for expressing recalcitrant proteins. [Read more of Application Note 15 at isotope.com.](#)



### Application Note 20

#### Effective Site-Specific Isotopic Labeling ( $^{15}\text{N}$ , $^{13}\text{C}$ Glycine; $^{15}\text{N}$ , $^{13}\text{C}$ Phenylalanine; $^{15}\text{N}$ Tryptophan) Expression Optimization Using BioExpress® 2000 Media

Kenneth C. Bonanno

Recombinant genes have been expressed in a variety of cellular systems for decades to generate protein reagents that are the potential targets for new small-molecule drugs. As these targets become more complex, researchers have developed innovative methods to study the structure of these proteins and the interactions with potential drugs. Cambridge Isotope Laboratories, Inc.'s (CIL) BioExpress® 2000 media incorporates isotope-labeled amino acids into recombinant protein expressed in baculovirus-infected insect cells to assist NMR structural studies (Strauss, et al., 2006). To use this labeled media most efficiently, expression conditions must be optimized at a small scale prior to production. [Read more of Application Note 20 at isotope.com.](#)



### Application Note 22

#### [2,3- $^{13}\text{C}$ ]-Labeled Aromatic Residues as a Means to Improving Signal Intensities and Kick-Starting the Assignment of Membrane Proteins by Solid-State MAS-NMR

Matthias Hiller, Victoria A. Higman, Stefan Jehle, Barth-Jan van Rossum, Werner Kühlbrandt and Hartmut Oschkinat

Over the past few years solid-state MAS-NMR has rapidly been developing into a structure determination technique for biological macromolecules.<sup>1-6</sup> Its advantages include the ability to study membrane proteins in their native lipid environment,<sup>7-10</sup> as well as making possible the study of non-soluble or non-crystallisable protein states, such as amyloid fibrils.<sup>11-15</sup> However, a prerequisite for structure determination is a high level of resonance assignment. There are numerous examples of small- and medium-sized proteins for which this has been possible,<sup>4,16-20</sup> but for large membrane proteins, such as the 281-residue outer membrane protein G (OmpG)<sup>21-22</sup> resonance assignment still remains a challenge. Signal overlap is an obvious problem, but fast longitudinal and transverse relaxation rates also contribute towards lower signal/noise ratios. Furthermore, membrane proteins can reduce the Q-factor of the coil, and the experimentalist is then left with the difficult task of balancing increased decoupling powers against possible sample heating which could lead to sample degradation. These problems result in lower signal intensities in proton-driven spin diffusion (PDS) spectra, however, they can be addressed using several different strategies, such as by improving coil design,<sup>23-25</sup> by developing spectral editing pulse sequences,<sup>26-27</sup> or by using novel labeling strategies.<sup>28-29</sup> It is this last approach, using a novel labeling strategy, which is presented here in this application note. [Read more of Application Note 22 at isotope.com.](#)

*Please visit [isotope.com](http://isotope.com) for additional bioNMR application notes.*



## Application Notes Summaries



### Application Note 25

#### Isotope Labeling of Alanine Methyl Groups on a Deuterated Background for NMR Studies of High-Molecular-Weight Proteins

Chenyun Guo, Raquel Godoy-Ruiz and Vitali Tugarinov

The state-of-the-art isotope-labeling schemes commonly employed for NMR investigations of high-molecular-weight proteins utilize selective incorporation of protons and  $^{13}\text{C}$  isotopes into methyl groups of Ile $\delta^1$ , Leu $\delta$  and Val $\gamma$  side chains in a highly deuterated environment (commonly referred to as “ILV labeling”) providing a large number of high-quality probes for NMR studies of protein structure and dynamics. Robust ILV labeling methods<sup>1,2</sup> and strategic location of ILV side-chains in the hydrophobic cores of protein structures<sup>3,4</sup> have turned ILV labeling into an indispensable tool for NMR studies of large proteins and macromolecular assemblies.<sup>5</sup> More than a decade ago, Gardner and Kay pioneered a technique for selective incorporation of protonated Ile $\delta^1$  methyl positions into protein molecules.<sup>6</sup> Subsequently, selective methyl labeling/protonation methods have been refined and extended to Leu $\delta$  and Val $\gamma$  methyl sites by the Kay group.<sup>7,8</sup> The introduction of methyl transverse relaxation optimized spectroscopy<sup>9</sup> (methyl-TROSY) has stimulated the development of improved labeling schemes.<sup>10,11</sup> Selective labeling of ILV methyl sites in large proteins on a deuterated background in synergy with methyl-TROSY techniques have had a significant impact on NMR studies of structure and dynamics of large protein assemblies up to ~800 kDa in molecular weight.<sup>12-15</sup> Nevertheless, the availability of only three ILV probes oftentimes presents a serious limitation for structural and dynamics studies of large proteins. Recent advances in methyl isotope labeling have focused on the extension of the ILV labeling methodology to methyl positions of alanines (Ala $\beta$ ). [Read more of Application Note 25 at isotope.com.](#)



### Application Note 39

#### Production of U-[ $^2\text{H}$ ], Thr- $\gamma^2$ [ $^{13}\text{CH}_3$ ] Labeled Proteins for Methyl-TROSY NMR

Lewis Kay, PhD

Isotope labeling has revolutionized the utility of biomolecular NMR spectroscopy, allowing the exploration of molecular interactions with high sensitivity and resolution.<sup>1,2</sup> Many different strategies are available, along with a wide array of NMR experiments that are optimized for the different labeling approaches. One scheme that has been shown to be particularly effective in studies of high-molecular-weight proteins involves labeling methyl groups as  $^{13}\text{CH}_3$  in an otherwise highly deuterated background<sup>3,4,5,6,7</sup> and exploiting a methyl-TROSY effect<sup>8</sup> that generates high-quality spectra. Applications to date have focused to a large extent on Ile, Leu and Val methyl probes,<sup>9</sup> as the precursors for these residues are commercially available and very easy to use. More recently, however, studies utilizing Met<sup>10,11</sup> and Ala<sup>12</sup> methyl groups have also emerged along with approaches for introducing methyls into key positions in the protein of choice. [Read more of Application Note 39 at isotope.com.](#)



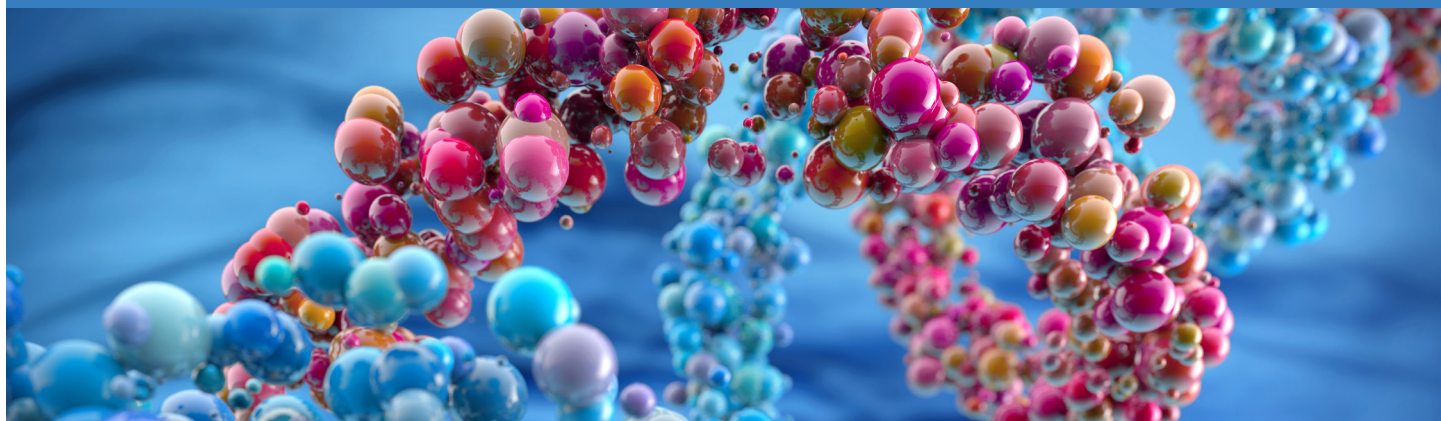
### Application Note 48

#### Stereospecific Leu/Val Methyl Labeling: An Important Technology for NMR Studies of High-Molecular-Weight Complexes

Rui Huang and Lewis E. Kay

The development of modern biomolecular solution NMR spectroscopy has paralleled innovations in labeling technologies. For example, early advances in heteronuclear double- and triple-resonance NMR spectroscopy were closely coupled to the emergence of methodologies for the effective uniform labeling of biomolecules with  $^{15}\text{N}$  and/or  $^{13}\text{C}$ .<sup>1,2</sup> Despite arguments that uniform  $^{13}\text{C}$  ( $^{13}\text{C}$ ) labeling would be prohibitively expensive, the high demand for  $^{13}\text{C}$ -labeled precursors has significantly lowered costs, and the preparation of biomolecules with  $^{13}\text{C}$  incorporation is most often the approach of choice for NMR studies.<sup>3</sup> As the size of the system studied increases, both the sensitivity and the resolution of the resulting spectra suffer, and this has led to the development of amide-TROSY-based experiments<sup>4</sup> that are significantly improved through the use of perdeuteration.<sup>5</sup> A powerful labeling scheme for studies of proteins over a wide range of molecular masses, but, in particular, for systems with aggregate masses in excess of 100 kDa involves  $^{13}\text{CH}_3$ -labeling in a highly deuterated background.<sup>6</sup> Initial schemes focused on Ile ( $\delta^1$ ), Leu and Val methyl groups, where for Leu and Val only one of the isopropyl methyls is  $^{13}\text{CH}_3$  (the other is  $^{12}\text{CD}_3$ ), but more recently precursors for the other methyl positions have become commercially available<sup>7-11</sup> so that it is possible to label the methyl group of choice. Once again, the availability of precursors stimulated the development of new NMR experiments, in this case those exploiting a methyl-TROSY effect,<sup>12</sup> which has resulted in applications involving protein complexes in excess of 1 MDa.<sup>13</sup> [Read more of Application Note 48 at isotope.com.](#)

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