



# *In-vivo* SILAM diet

Stable isotopic labelling is the most reliable and accurate method for quantitative proteomics and metabolomics. Proteomics studies on uniformly  $^{15}\text{N}$ -labelled rats have been previously reported using  $^{15}\text{N}$ -labelled algae as protein source for Stable Isotope Metabolic Labelling in Mammals (SILAM).<sup>1</sup>

Silantes provides SILAM diets for *in-vivo* labelling of mouse, but also for other model organisms such as fly<sup>2</sup> and worm<sup>3</sup> (see literature in the footnote). To see our product portfolio of *in-vivo* SILAM diets, scan the QR-code in the top right corner.

## Silantes $^{15}\text{N}$ -SILAM Diets for *in-vivo* labelling of mice

Silantes has developed a  $^{15}\text{N}$ -diet in cooperation with the group of Professor Chris Turck, Max-Planck-Institute of Psychiatry as a kit containing a  $^{15}\text{N}$ -labelled "heavy" diet (B) and unlabelled "light" diet (A).<sup>4</sup> It is an artificial feed using Harlan components and a hydrolysate of the bacteria *Ralstonia eutropha* as  $^{15}\text{N}$ -protein source. Feeding experiments showed that the mice accepted bacterial-based protein better than algae-based protein.



Figure 1: Silantes  $^{15}\text{N}$ -labelled mice feed

After the metabolic labelling (feeding) of the mice according to the scheme in figure 2 or figure 3, the mice are sacrificed. Differences in the protein patterns are determined in analogy to the established SILAC approach in cell culture (see literature for Silantes *in vitro* SILAC).

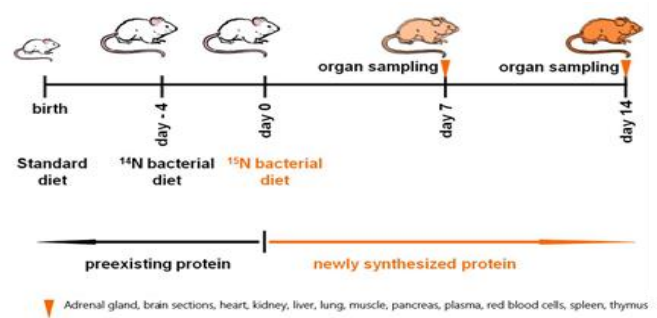


Figure 2: Partial *in-vivo* labelling for global protein turnover

<sup>1</sup> Daniel B. McClatchy, Meng-Qiu Dong, Christine C. Wu, John D. Venable, and John R. Yates (2007).  $^{15}\text{N}$  Metabolic Labeling of Mammalian Tissue with Slow Protein, *Journal of Proteome Research*, 6(5), 2005-2010.

<sup>2</sup> Sury, M. D., Chen, J. X., & Selbach, M. (2010). The SILAC fly allows for accurate protein quantification *in vivo*. *Molecular & cellular proteomics : MCP*, 9(10), 2173–2183. <https://doi.org/10.1074/mcp.M110.000323>

<sup>3</sup> Larance, M., Bailly, A. P., Pourkarimi, E., Hay, R. T., Buchanan, G., Coulthurst, S., Xirodimas, D. P., Gartner, A., & Lamond, A. I. (2011). Stable-isotope labeling with amino acids in nematodes. *Nature methods*, 8(10), 849–851. <https://doi.org/10.1038/nmeth.1679>

<sup>4</sup> Zhang, M.D. Filiou, G. Chen, H. Heumann, Chris W. Turck (2008). Biomarker discovery in a mouse model of trait anxiety using  $^{15}\text{N}$  metabolic labeling, Poster HUPO Amsterdam.



# *In-vivo* SILAM tissue

A variation of the *in-vivo* SILAM-approach is the “spike-in” approach.

## SILAM spike-in using Silantes $^{15}\text{N}$ -labelled mouse tissue

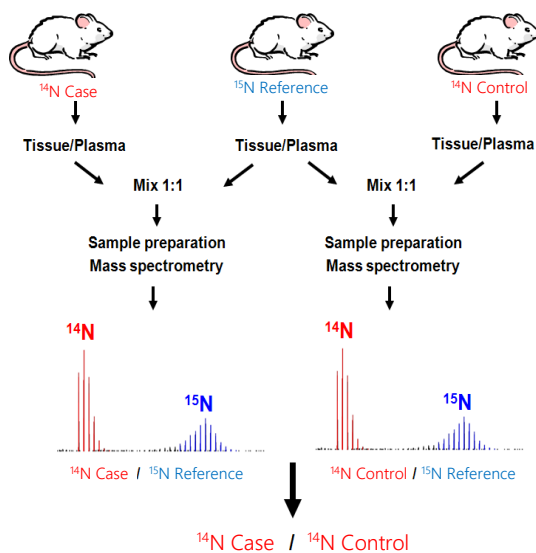


Figure 3: Uniform *in-vivo* labelling of SILAM mouse

Figure 2 shows the SILAM spike-in workflow: Differences in protein patterns of unlabelled case tissue with respect to unlabelled control tissue can be quantified by “spiking-in” a  $^{15}\text{N}$ -labelled reference tissue.

This  $^{15}\text{N}$  reference tissue can be obtained from Silantes directly, as frozen material or lyophilized (see literature for Silantes lyophilized tissue for SILAM spike-in).

To see our product portfolio of SILAM mouse tissues, scan the QR-code in the top right corner.

### Short outline of the procedure:

The isotopically labelled “heavy” reference tissue is mixed with the unlabelled “light” case and control tissue, respectively. The proteomes of the case/reference-mix and control/reference-mix are isolated, digested and subjected to LC-MS. Therefore, the two peptide amount ratios can be determined in analogy to the *in-vivo* SILAC-workflow to determine the case/control ratio (see Silantes *in-vivo* SILAC literature).

In addition to spiking-in the  $^{15}\text{N}$ -tissue as reference material for relative protein quantification by mass spectrometry, the SILAM approach is used in further applications such as

- Partially labelled mouse specimens for global protein turnover analysis by mass spectrometry (see figure 2)<sup>5</sup>
- Labelled metabolite standards for molecular structure confirmation
- Biomarker discovery and verification

<sup>5</sup> Zhang, Reckow S, Webhofer C, Boehme M, Gormanns P, Egge-Jacobsen WM, Turck CW. (2011) Proteome scale turnover analysis in live animals using stable isotope metabolic labeling. *Anal Chem* 83:1665-1672.