Silantes
Stable Isotope Labeled Biomolecules

Scan the QR-Code to see our product catalogue of SILAC Diets.

In-vivo SILAC diets

In vitro SILAC is a well-established approach for quantification of proteins in cell culture.[1] The same concept can also be applied to living organisms (*in-vivo* SILAC). The latter approach allows determination of protein patterns of all tissues of an organism with respect to a reference organism. This approach has been successfully applied to the analysis of mouse organs.[2]

Prerequisite for the application of the *in-vivo* SILAC approach is the availability of an appropriate SILAC diet in which all lysins are replaced by ^{13}C-lysine.

Silantes provides SILAC diets for *in-vivo* labelling of mouse, but also for other model organisms such as fly[3] and worm[4]. To see our product portfolio of *in-vivo* SILAC diets, scan the QR-code in the top right corner.

Silantes SILAC Diets for *in-vivo* labelling of mice[5]

Mice are fed with Silantes SILAC mouse feed according to the scheme in Figure 2 on the next page for about 90 days requiring about 800 g Silantes mice feed.

^{13}C-lysine labelled mice feed (Figure 1) has been developed in cooperation with the group of Prof. Matthias Mann, Max-Planck-Institute of Biochemistry as a kit containing labelled and unlabelled mouse feed. The kit consists of ^{13}C-lysine-labelled "heavy" diet (B) and unlabelled "light" diet (A). The feed is an artificial, amino acid-based feed using Harlan components.

Figure 1: Silantes ^{13}C-lysine mice feed

After the metabolic labelling (feeding) of the mice according to the scheme in Figure 2, 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). Instead of ^{13}C-lysine labelled feed, other isotopic formulations are also available on request, e.g. ^{13}C,^{15}N-labelled feed.

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[1] Ong, S.E., Blagoev, B., Kratchmarova, I., Kristensen, D.B., Steen, H., Pandey A., and Mann, M. (2002). Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics, Mol.Cell. Proteomics 1, 376–386.

[2] Ong, S.E., and Mann, M. (2006). A practical recipe for stable isotope labelling by amino acids in cell culture (SILAC). Nat. Protoc. 1, 2650–2660.

[3] 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

[4] 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

[5] Krueger M., Moser M., Ussar S., Thievessen I., Luber Ch.A, Forner F., Schmidt S., Zanivan S., Faessler R. and Mann M.(2008). SILAC Mouse for Quantitative Proteomics Uncovers Kindlin-3 as an Essential Factor for Red Blood Cell Function, Cell 134, 353–364.

Scan the QR-Code to see our product catalogue of SILAC Mouse Tissues.

In-vivo SILAC tissue

A variation of the *in-vivo* SILAC-approach is the "spike-in" approach.

SILAC spike-in using Silantes $^{13}C_6$-Lysine-labelled mouse tissue

Figure 2 shows the SILAC spike-in workflow: Differences in protein patterns of unlabelled tissue (A) with respect to unlabelled tissue (B) can be quantified by "spiking-in" a ^{13}C-labelled reference tissue (R). This ^{13}C-lysine reference tissue can be obtained from Silantes directly, as frozen material or lyophilized (see literature for Silantes lyophilized tissue for SILAC spike-in). To see our product portfolio of SILAC mouse tissues, scan the QR-code in the top right corner.

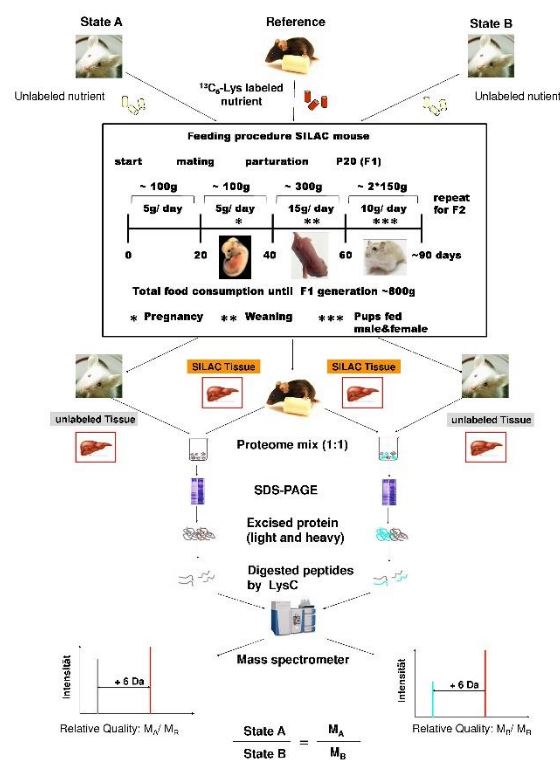

Figure 2:in-vivo SILAC-mouse workflow

<u>Short outline of the procedure:</u>

The isotopically labelled "heavy" reference tissue (R) is mixed with the unlabelled "light" tissues (A) and (B), respectively. The proteomes of the (A)(R)-mix and (B)(R)-mix are isolated, digested and subjected to LC-MS as shown in the workflow. Therefore, the two peptide amount ratios (A/R) and (B/R) can be determined.

Calculating the ratio (A/R) : (B/R) cancels out the reference amount (R) and yields the ratio of peptides (A/B). The stable isotopically labelled mouse tissue (R) is used as a standard, permitting normalizing (A) with respect to (B), accounting for differences in the isolation procedure in the mixtures (A/R) and (B/R) without affecting the peptide ratios (A/B). (Strain (R) must not necessarily be the same as strain (A) and (B).)

Conclusion:

The customer is free in regard to the choice of strain used for the experiment but can use the Silantes $^{13}C_6$-Lysine-labelled mouse tissue as a reference which is spiked into tissue (A) and (B).