Stable Isotope labeled Compounds for in-vitro SILAC Experiments

In vitro SILAC (Stable Isotope Labeling of Amino Acids in Cell Culture) has been proven a powerful technique for quantitative proteomics in cell culture. The method is robust and provides accurate results.[1]

The in-vitro SILAC workflow:

Figure 1 shows the workflow of the SILAC procedure to quantitatively determine differences in the protein pattern of two cultures:

Step 1: Culture A ("light") is supplemented with unlabeled amino acids, whereby culture B ("heavy) is supplemented with labeled amino acids. As an example, in culture B, the $^{12}C_6$-lysine is substituted by $^{13}C_6$-lysine.

Step 2: Cells from both cultures are mixed in a 1:1 ratio. The proteins are isolated and digested with Lys-C, a protease which specifically cleaves at lysines.

Step 3: The proteolytic cleavage creates corresponding pairs of peptides stemming from culture A and B, differing by a molecular weight of 6 Da due to the molecular weight difference of the terminal $^{13}C_6$-lysine. The ratio of the amount of "light" and "heavy" peptides is determined by mass spectrometry.

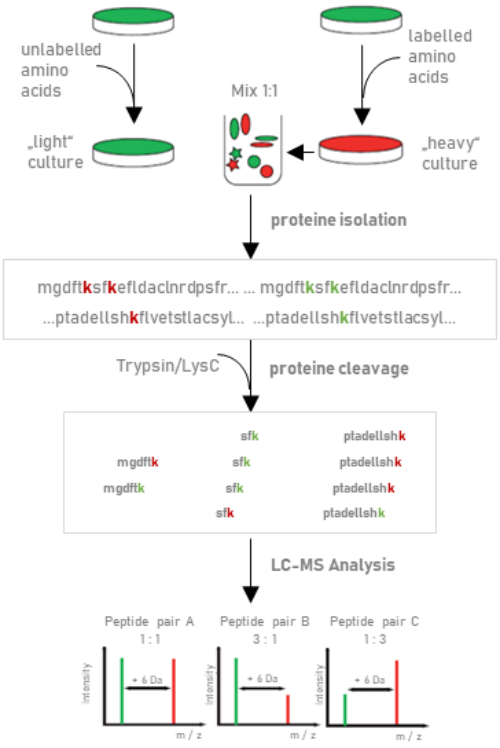

Figure 1: In-vitro SILAC workflow

Silantes Components for *in-vitro* SILAC Experiments

Silantes offers all components that are necessary for a SILAC experiment. Each component is in a prepared sterile solution and ready for use. The components are available as individual products or in a kit. Each kit consists of:

- 2 x 500 mL Silantes SILAC DMEM or RPMI media free of the amino acids lysine and arginine
- 2 x 50 mL Silantes dialyzed FBS
- Unlabeled L-lysine and L-arginine
- SILAC amino acids L-lysine and L-arginine

[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.

E-Mail: sales@silantes.com
Tel.: 0049 (0) 89 / 500 941 – 0
Web: www.silantes.com

High Quality of Silantes SILAC Components

The SILAC amino acids are available in all isotopic combinations. We guarantee an isotopic enrichment of > 98 atom % with a chemical purity of > 95 %. The isotopic purity is tested by mass spectrometry, whereas the chemical purity is tested by HPLC.

Figure 2 shows the growth kinetics of a model mammalian cell line using Silantes SILAC media and different labeling patterns of the SILAC L-lysines.

The experiment shows that the cells grow well on the Silantes SILAC components.

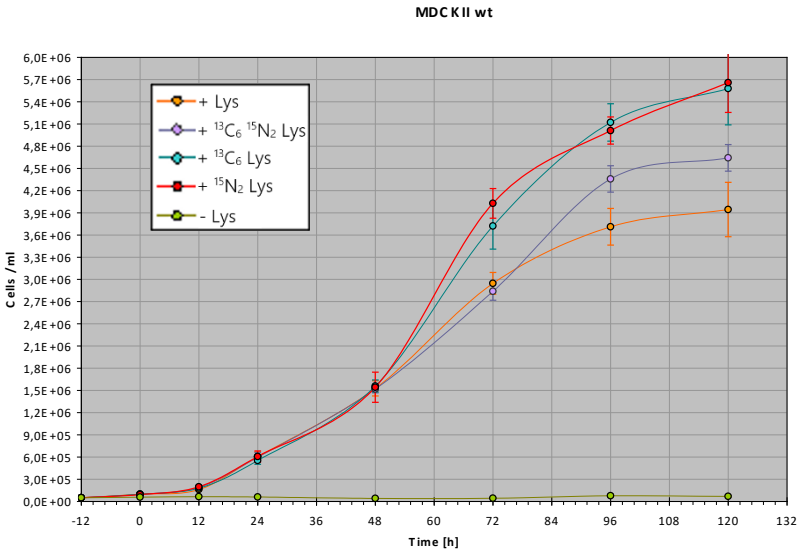

Figure 2: Kinetics of MDCK cells on Silantes SILAC media

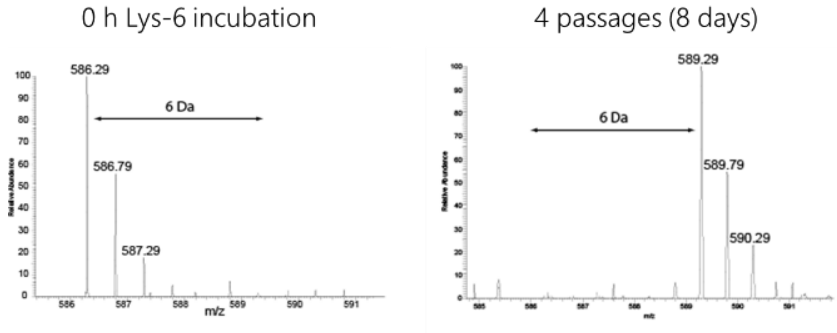

Figure 3: Incorporation of Silantes Lys-6

Figure 3 shows the incorporation of $^{13}C_6$-lysine in an actin peptide (molecular weight = 586 Da) during the preparation of the "heavy" culture for a SILAC experiment

A comparison of the 586 Da peak at t = 0 hours stemming from the unlabeled actin peptide and the 589 Da peak at t = 8 days stemming from the corresponding labeled actin peptide indicates that the cell culture is fully labeled after 8 days (4 passages). That the nominal difference of the peaks is 3 Da (and not 6 Da) is due to the fact that the ratio m/z (x-coordinate) is 2 (instead of 1).

E-Mail: sales@silantes.com
Tel.: 0049 (0) 89 / 500 941 – 0
Web: www.silantes.com