Stable Isotope labeled Compounds for in-vitro SILAC: CTAP with DAP

The SILAC approach is a powerful tool in quantitative proteomics of cell cultures.[1] Two cell populations are cultured in separate media, one containing unlabeled and one $^{13}C/^{15}N$-labeled arginine or lysine. The two cell populations are then combined for mass spectrometry analysis facilitating relative quantitation of a great number of proteins.

The SILAC method has limited applicability in multicellular contexts as cultures usually have to be grown in isolation. A new technique for the investigation of cell-cell interactions is the cell type-specific labeling using amino acid precursors (CTAP).[2] Instead of the essential amino acid itself, a stable isotope (SI)-labeled substrate or precursor is used. By overexpressing an enzyme that synthesizes the desired amino acid from its precursor, a metabolic incorporation of the label into a specific cell type is achieved.[3]

Silantes High Quality Components for Cell Type-Specific Labeling using Amino Acid Precursors

Silantes provides SI-labeled diaminopimelic acid (DAP), which is a precursor for the SILAC amino acid lysine. By transfecting distinct cell populations with the gene encoding the enzyme diaminopimelate decarboxylase (DDC), these cells gain the ability to grow on DAP instead of lysine.

Silantes offers ^{13}C-, ^{15}N- and $^{13}C^{15}N$-labeled DAP in powder form, which is produced by fermentation. We guarantee an isotopic enrichment of > 97 atom % and a chemical purity of > 95 %. The isotopic and chemical purities are confirmed by mass spectrometry and HPLC, respectively.

Product	Mass Shift	Quantity	Article Number
^{13}C-labeled L-Diaminopimelic acid powder	7 Dalton	100mg 1g	220203900 220204100
^{15}N-labeled L-Diaminopimelic acid powder	2 Dalton	100mg 1g	220303900 220304100
^{13}C ^{15}N-labeled L-Diaminopimelic acid powder	9 Dalton	100mg 1g	220603900 220604100

1 Ong, S. E., Blagoev, B., Kratchmarova, I., Kristensen, D. B., Steen, H., Pandey, A., 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(5):376-386.

2 Gauthier, N., Sander, C., Miller, M., (2014), Cell selective proteome labeling, WO 2014039643.

3 Gauthier, N. P., Soufi, B., Walkowicz, W. E., Pedicord, V. A., Mavrakis, K. J., Macek, B., Gin, D. Y., Sander, C. and Miller, M. L. (2013). Cell-selective labeling with amino acid precursors for proteomic studies of multicellular environments. Nat Methods 10(8), 768-773.

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

Stable Isotope labeled Compounds for in-vitro SILAC: CTAP with DAP

Applications of the CTAP Approach using SI-labeled DAP

In cell-of-origin secretome studies with lysine-auxotrophic cells, biomarker and cell-cell communication analysis can be performed in co-culture by metabolic labeling with DAP as a substrate (Figure 1).

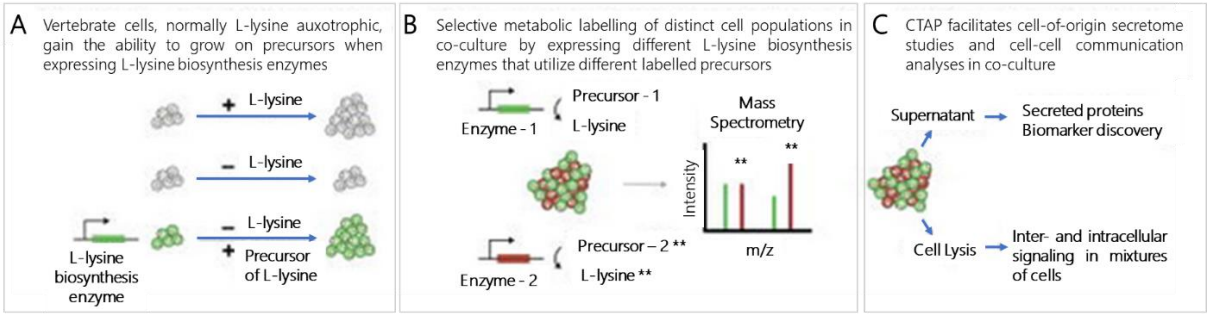

Figure 1: Cell type-specific labeling using amino acid precursors (CTAP). Method illustration from Gauthier et al.[2,3]

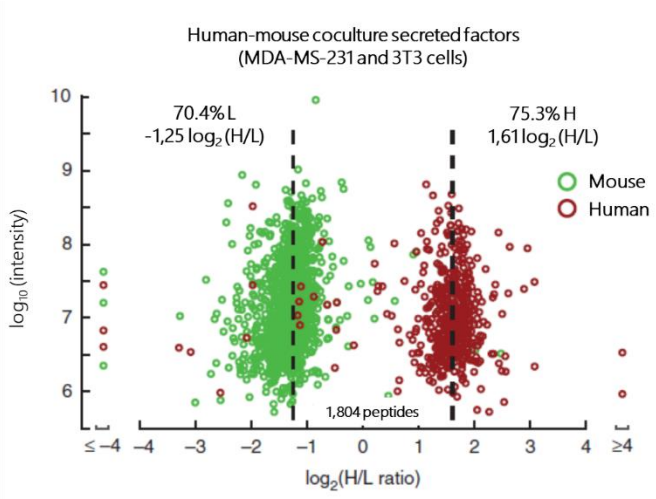

Figure 2: CTAP approach for secreted proteins. LC-MS/MS analysis of proteins in the supernatants of DDC-expressing mouse (3T3) and lyr-expressing human (MDA-MB-231) cells co-cultured in SI-labeled D-Lys (H = heavy) and unlabeled DAP (L = light). Illustration from Gauthier et al.[3]

Figure 2 shows an example of a cell-of-origin secretome study using the method of Gauthier et al..[3] A mouse cell line modified to express DDC for growth on DAP together with a human cell line capable of growing on D-lysine (expressing lysine racemase) were grown in co-culture on labeled D-lysine and unlabeled DAP. Nearly all proteins secreted from both cell lines could be distinguished by the difference in label illustrating the aptitude of CTAP for multicellular analysis. Additional applications of these studies are possible with the new availability of SI-labeled DAP.

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