



**Schematic of SILAC workflow.** A549 cells adapted to DMEM were grown for six passages (10 days) using SILAC DMEM (PN89983) containing 0.1 mg/mL heavy  $^{13}\text{C}_6$  L-lysine-2HCl or light L-lysine-HCl supplemented with 10% dialysed FBS. After 100% label incorporation,  $^{13}\text{C}_6$  L-lysine-labelled cells were treated with 5  $\mu\text{M}$  camptothecin (Sigma, St. Louis, PNC9911) for 24hr. Cells from each sample (light and heavy) were lysed using Thermo Scientific M-PER Mammalian Protein Extraction Reagent (PN78501). Samples were normalised for protein concentration using the Thermo Scientific Pierce BCA protein assay (PN23225), and 50mg of each sample were equally mixed before 4% to 20% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Gels were stained with Thermo Scientific GelCode Blue stain reagent (PN24592) and proteins were digested and alkylated using the Thermo Scientific Pierce in-gel Tryptic digestion kit (PN89871) before analysis using an LTQ Orbitrap hybrid mass spectrometer.

### SILAC protein quantitation kits and reagents, Thermo Scientific Pierce

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NEW

PN

For quantitative analysis of differential protein expression.

- Characterisation of proteins involved in stem cell differentiation using stem cell specific kits
- Quantitative analysis of relative changes in protein abundance from different cell treatments
- Quantitative analysis of proteins for which there are no antibodies available
- Protein expression profiling of normal vs. disease cells
- Identification and quantification of hundreds to thousands of proteins in a single experiment
- 100% label incorporation into proteins of living cells
- Media deficient in both L-lysine and L-arginine, allowing for more complete proteome coverage through dual amino acid isotope labelling
- Labels expressed in a wide variety of mammalian cell lines adapted to grow in DMEM or RPMI 1640 medium, including HeLa, 293T, COS7, U2OS, A549, A431, HepG2, NIH 3T3, Jurkat and others

Stable isotope labelling using amino acids in cell culture (SILAC) is a powerful method to identify and quantify relative differential changes in complex protein samples.

The SILAC method uses *in vivo* metabolic incorporation of 'heavy'  $^{13}\text{C}$ - or  $^{15}\text{N}$ -labelled amino acids into proteins followed by mass spectrometry (MS) analysis for accelerated comprehensive identification, characterisation and quantitation of proteins.

SILAC requires growing mammalian cells in specialised media supplemented with light or heavy forms of essential amino acids; e.g.,  $^{13}\text{C}_6$  and  $^{13}\text{C}_5$  L-lysine, respectively. A typical experiment involves growing one cell population in medium containing light amino acids (control), while the other population is grown in the presence of heavy amino acids (experimental). The heavy and light amino acids are incorporated into proteins through natural cellular protein synthesis. After alteration of the proteome in one sample through chemical treatment or genetic manipulation, equal amounts of protein from both cell populations are then combined, separated by SDS-polyacrylamide gel electrophoresis and digested with trypsin before MS analysis. Because peptides labelled with heavy and light amino acids are chemically identical, they co-elute during reverse-phase column pre-fractionation and, therefore, are detected simultaneously during MS analysis. The relative peak intensities of multiple isotopically distinct peptides from each protein are then used to determine the average change in protein abundance in the treated sample (see Figure).

Please note: all media for SILAC are minus L-Lysine and L-Arginine.

Catalogue No	Description
<b>PN89982</b>	<b>Pierce SILAC protein quantitation kit – RPMI 1640</b> Includes: RPMI Media for SILAC, 2 x 500mL Dialysed FBS, 2 x 50mL $^{13}\text{C}_6$ L-Lysine $\cdot$ 2HCl, 50mg L-Lysine $\cdot$ 2HCl, 50mg L-Arginine $\cdot$ HCl, 2 x 50mg
<b>PN89983</b>	<b>Pierce SILAC protein quantitation kit – DMEM</b> Includes: DMEM Media for SILAC, 2 x 500mL Dialysed FBS, 2 x 50mL $^{13}\text{C}_6$ L-Lysine $\cdot$ 2HCl, 50mg L-Lysine $\cdot$ 2HCl, 50mg L-Arginine $\cdot$ HCl, 2 x 50mg
<b>PN89984</b>	<b>RPMI media for SILAC, 500mL</b>
<b>PN89985</b>	<b>DMEM media for SILAC, 500mL</b>
<b>PN88215</b>	<b>DMEM:F12 (1:1) media for SILAC, 500mL</b>
<b>PN89986</b>	<b>Dialysed FBS, 50mL</b>
<b>PN89987</b>	<b>L-Lysine <math>\cdot</math> 2HCl, 50mg</b>
<b>PN89988</b>	<b><math>^{13}\text{C}_6</math> L-Lysine <math>\cdot</math> 2HCl, 50mg</b>
<b>PN89989</b>	<b>L-Arginine <math>\cdot</math> HCl, 50mg</b>
<b>PN89990</b>	<b><math>^{13}\text{C}_6</math> <math>^{15}\text{N}_4</math> L-Arginine <math>\cdot</math> HCl, 50mg</b>
<b>PN88209</b>	<b><math>^{13}\text{C}_6</math> <math>^{15}\text{N}_2</math> L-Lysine <math>\cdot</math> 2HCl, 50mg</b>
<b>PN88210</b>	<b><math>^{13}\text{C}_6</math> L-Arginine <math>\cdot</math> HCl, 50mg</b>
<b>PN88211</b>	<b>L-Proline, 115mg</b>
<b>PN88214</b>	<b>Phenol red free MEM for SILAC, 500mL</b>