SILAC Reagents (Trinkle Lab Protocol October 2008; www.trinklelab.com)

1. DMEM or RPMI minus Arg, Lys and Met

Source (North America): AthenaES

Order No: 0500-03 (95 USD/L, minimum order 5L)

Note: *Other sources include Invitrogen, Wisent and Biowest), including powdered media that you resuspend.*

2. Dialyzed Fetal Calf Serum

Source: Invitrogen

Order No: 26400-044 (500 ml) 472.25 CAD

3. Standard Amino Acids (for R0K0 media)

Source: Sigma

Order No: L-Arginine (A8094, 25g, 25.50 CAD), L-Lysine (L8662, 25g, 20.30 CAD), L-Methionine (M5308, 25g, 33.60 CAD)

Note: prepare as stock solutions in PBS and store in 0.5 ml aliquots at -20C. Add 0.5 ml aliquot of each when preparing R0K0 media (and also add a 0.5 ml aliquot of Met0 when preparing R6K6, R6K4 and R10K8 media).

Arg0: 84 mg/ml stock
Lys0: 146 mg/ml stock
Met0: 30 mg/ml stock

4. Isotopically-Labeled Amino Acids (for R6K6, R6K4 and R10K8 media)

Source: Cambridge Isotope Lab (CIL; North America; www.isotope.com).

Order No./Price (for larger amounts):

Amino Acid	Short Name	Cat. No.	Pack size	Cost (CAD)
L-Arginine:HCl (U-13C6, 98%)	R6	CLM-2265	0.5g	1692
L-Arginine:HCl (U-13C6, 98%; 15N4, 98%)	R10	CNLM-539	0.5g	2200
L-Lysine-2HCl (4,4,5,5-D4, 96-98%)	K4	DLM-2640	0.5g	470
L-Lysine-2HCl (U-13C6, 98%)	K6	CLM-2247	0.5g	1600
L-Lysine-2HCl (U13C6, 98%; 15N2, 98%)	K8	CNLM-291	0.5g	1040

Note: 0.5g of arginine will yield 12 aliquots (for 12 x 500 ml bottles of media) and 0.5g of lysine will yield 7 aliquots

Prepare isotopes as stock solutions in PBS and store in 0.5 ml aliquots at -20C. There is no need to weigh out the amount (and risk losing expensive reagents!)…simply add the correct volume of solvent to the vial, assuming the amount stated on the vial to be correct (We have had no problems with isotopes from CIL). Add a 0.5 ml aliquot of each when preparing labeled media.

Arg6 or Arg10: approx. 84 mg/ml stock (dissolve 0.5g of Arg in 6 ml of PBS and freeze 12 x 0.5 ml aliquots…sufficient for 12 x 500 ml bottles of DMEM)
Lys4, Lys6 or Lys8: approx. 146 mg/ml stock (dissolve 0.5g of Lys in 3.5 ml of PBS and freeze 7 x 0.5 ml aliquots…sufficient for 7 x 500 ml bottles of DMEM)

Note: all amino acids (unlabeled or labeled) can be stored at room temperature in powder form before you dissolve them. The above amino acid concentrations are based on the formula of normal RPMI (Invitrogen Cat no. 21875). Some labs specifically use a suboptimal concentration of Arg or Lys in order to to prevent the cells from converting the isotope labels into other, nonessential, amino acids such as proline. See http://www.pil.sdu.dk/silac_media.htm for a discussion of this topic.

***If you only plan to do 1 or 2 experiments, you can order smaller amounts of the isotopes:*

Order No./Price (for smaller amounts):

Amino Acid	Short Name	Cat. No.	Pack size	Cost (CAD)	Pack size	Cost (CAD)
L-Arginine:HCl (U-13C6, 98%)	R6	CLM-2265	0.25g	995	0.1g	585
L-Arginine:HCl (U-13C6, 98%; 15N4, 98%)	R10	CNLM-539	0.25g	1287	0.1g	770
L-Lysine-2HCl (4,4,5,5-D4, 96-98%)	K4	DLM-2640	-	-	-	-
L-Lysine-2HCl (U-13C6, 98%)	K6	CLM-2247	0.25g	1016	0.1g	610
L-Lysine-2HCl (U13C6, 98%; 15N2, 98%)	K8	CNLM-291	0.25g	522	0.1g	310

Note: 0.25g of arginine will yield 6 aliquots (for 6 x 500 ml bottles of media), and 0.25g of lysine will yield 3 aliquot (for 3 x 500 ml bottles of media).
0.1g of arginine will yield 2 aliquots (for 2 x 500 ml bottles of media) with some left over, and 0.1g of lysine will yield 1 aliquot (with some left over).
**** there will be leftover lysine when 0.25g or 0.1g is solubilized, so freeze it and combine it with future leftovers to make up another full aliquot.***

5. Cell Dissociation Buffer (for passaging cells)

Source: Invitrogen

Order No./Price: Cat No. 13151-014 (100 ml); 28.30 CAD

Note: Do not use Trypsin-EDTA to passage cells in SILAC medium (may contain amino acids).

**The above reagents are everything that you will need to prepare SILAC media and passage your cells. You may add other antibiotics if necessary (e.g. we use G418 for our stable GFP cell lines). Where appropriate, you may also supplement your media with L-glutamate. Cells should be completely labelled after 5 cell cycles. We normally seed cells at 20% confluence in the SILAC medium and let them grow to 80% confluence. We then seed the cells again in new plates in SILAC medium at 20% and harvest the cells when they are 80% confluent.

Please note that some cell lines are not viable or grow poorly in SILAC medium. It is therefore advisable to first test your cells in SILAC medium containing unlabeled amino acid (R0K0) and use the expensive labeled media only when you are happy with the cell growth. Poor growth is probably due to the use of dialyzed fetal calf serum, which lacks some small molecules important for cell growth.

Preparing SILAC Media

To 500 ml of liquid media, add:

50 ml dialysed fetal calf serum
5.5 ml Pen/Strep (and/or other antiobiotics, if desired)
0.5 ml Met0 stock
0.5 ml Arg stock (R0, R6 or R10)
0.5 ml Lys stock (K0, K6, K4 or K8)

Mix well. Filter through 0.22µm filter using a suction pump and store at 4ºC.

Which Labels Should I Use?

To do a **double labeling experiment** (comparing 2 different populations of cells), use:

R0K0 vs. R6K6

You'll need one 500 ml bottle of 500 ml R0K0 DMEM and one 500 ml bottle of R6K6 DMEM. If using 14 cm dishes, 12 ml of media is sufficient to cover the cells. That means that these 2 bottles of media are enough for approximately 40 x 14 cm dishes of cells (a bit less when you account for the media you need to split the cells initially). We routinely use 10 x 14 cm dishes per IP SILAC experiment, so these *2 bottles of media are sufficient for at least 3 large-scale double-encoding IP SILAC experiments.*

To do a **triple labeling experiment** (comparing 3 different populations of cells), use:

R0K0 vs. R6K4 vs. R10K8

You'll need one 500 ml bottle of 500 ml R0K0 DMEM, one 500 ml bottle of R6K4 DMEM and one 500 ml bottle of R10K8 DMEM. These *3 bottles of media are sufficient for at least 3 large-scale triple-encoding IP SILAC experiments.*

How Much Material?

As mentioned above, we typically use 10 x 14 cm dishes of cells for each condition in an IP SILAC experiment. You should see visible bands on the gel by Coomassie staining, which puts you in a range of 2-5 ug of total protein on the gel (but more is always better!) Here's an example: