Cell Culture in SILAC media

(Boisvert Lab, December 2013)

Material:

We use the following material to make our SILAC media. We have tried several cheaper alternative which often resulted in cells not growing and/or dying rapidly. We have also tried different prepackaged kit which are often 2-3 times the price with no better results.

Because the serum is dialyzed, it is possible that some growth factor will be missing. Some cell lines grow better in the presence of insulin, EGF, etc… and these can easily be supplemented as needed.

DMEM + 4.5g/L glucose, - L-Arg, - L-Lys, -L-Glutamine, - Sod. Pyruvate
Invitrogen A14431-01

FBS triple dialyzed
Invitrogen 26400-044

GlutaMAX
Gibco 35050-061

Pen/Strept (optional, but recommended):

Sodium Pyruvate (optional):

Hepes (optional):

Normal and isotopic amino acids

L-Arginine ‘Light’, R0: Sigma A6969
L-Lysine ‘Light’, K0: Sigma A8662

Stable isotopes from Cambridge Isotope Laboratories, inc:

L-Arginine ‘Medium’, R6: CLM-2265
L-Lysine ‘Medium’, K4: DLM-2640

L-Arginine ‘Heavy’, R10: CNLM-539
L-Lysine ‘Heavy’, K8: CNLM-291

Arginines is supposed to be resuspended at 84 mg/ml stock, and lysine at 146 mg/ml stock. For convenience, we resuspend 250 mg of arginine in 3 ml of PBS to make 6 aliquots of 500 ul and 250 mg of lysine in 2ml of PBS to make 4 aliquots of 500 ul.

Making a bottle of SILAC medium:

Only use dialyzed serum. Otherwise, the 'normal' amino acids present in the serum will completely dilute out the isotopes and waste the bottle.

Add 50 ml of dialyzed serum to 500 ml of DMEM –arg-lys, along with 5 ml of GlutaMAX, Pen/Strept, Sodium Pyruvate and Hepes, as needed.

Add 500 ul of Arginine and 500 ul of Lysine stock solutions.

Mix well and filter using a 500 ml Stericup (Millipore) to ensure sterility.

Cell culture for incorporation of labelled amino acids

NOTE: To ensure that your cell lines will tolerated the growing conditions, we strongly suggest to initially do a few passages in 'light' (R0K0) media before using the more expensive amino acids.

It is recommended to grow your cells for at least 6 population doubling to ensure near 100% incorporation of the isotopic amino acids before you can do your experiment. In practice, we found that 4 passages were sufficient to have near full incorporation.

We normally dilute cells that are 80% confluent 1/3 into a new plate, and let it grow back to 80%. This constitute 1 passage.

We recommend to perform these passages initially using a small volume to save costs. For example, if you need 5 large 150mm petri dishes:

1) Take one 60mm petri dish at 80% confluence, trypsinize.
   Dilute 1/3 into a 60mm petri dish in 3 ml SILAC media. This is passage 1.

2) After it reaches 80% confluence, trypsinize.
   Dilute 1/3 into a 60mm petri dish in 3 ml SILAC media. This is passage 2.

3) After it reaches 80% confluence, trypsinize.
   Dilute 1/2 into a 100mm petri dish in 8 ml SILAC media. This is passage 3.

4) After it reaches 80% confluence, trypsinize.
   Dilute 1/2 into a 150mm petri dish in 18 ml SILAC media. This is passage 4.
   Your cells should now be fully incorporated.

5) After it reaches 80% confluence, trypsinize.
   Seed cells in five 150mm petri dishes in 18 ml SILAC.
   Let them grow to 80% confluence.

   This will use up (3+3+8+18+(18X5)) = 122 ml of SILAC media for each condition.

A note regarding arginine to proline conversion

Recent studies using stable isotope labeling with amino acids in culture (SILAC) in quantitative proteomics have made mention of the problematic conversion of isotope-coded arginine to proline in cells. The resulting converted proline peptide divides the heavy peptide ion signal causing inaccuracy when compared with the light peptide ion signal.

It was demonstrated that by adding an additional 200 mg/liter of proline, it is possible to completely prevent this conversion, thus allowing correct interpretation of light and heavy peptide ratios (Bendall *et al.*, MCP 2008).

Another proven alternative is to reduce the amount of arginine to 17-21 mg/L to prevent conversion.

While this has been reported to be an issue for some specific cell lines or organisms, we have rarely found this to be a problem for our cell lines and we have chosen to not adjust the amount of arginine or proline unless we detect a problem with conversion.