Preparing medium for metabolic SILAC labeling

This document describes a typical preparation of culture medium in order to perform a metabolic labeling experiment, such as SILAC.

Quick links

Reagents and materials

You should choose which labeling schemes you would like to apply and order reagents below based on this. Specifically, you do not need the 'medium' amino acids if you only plan to do light and heavy conditions, so in this case, you would not order the lysine +4 or the arginine +6.

- 1.5mL snap-lock tubes (Thermo Scientific, CAT#05-402-25)
- DMEM without arginine, lysine, glutamine (Silantes, CAT#280001200)
- GlutaMAX, stable glutamine stock (Thermo Scientific, CAT#35050061)
- Dialyzed FBS (Silantes, CAT#281000800)
- MEM Non-essential Amino Acids (Thermo Scientific, CAT#11140050)
- Lysine +0 (Silantes, CAT#211004102)
- Arginine +0 (Silantes, CAT#201004102)
- Lysine +4 (Silantes, CAT#211104113)
- Arginine +6 (Silantes, CAT#201204102)
- Lysine +8 (Silantes, CAT#211603902)
- Arginine +10 (Silantes, CAT#201604102)
- Proline (Sigma, CAT#P5607)
- Nalgene Rapid-Flow filter units, 500mL, 0.2um filter (VWR, CAT#73520-984)

Solution recipes

- lysine (500X stock) - 1g powder in 14mL of water
- arginine (500X stock) - 1g powder in 24mL of water
- proline (500X stock) - 1g powder in 10mL of water

The amino acid stocks should be aliquoted (1mL each) and stored at -20C until use.

Protocol

1. Pour 500mL of DMEM into the top of a bottle-top filter unit.
2. Filter the media down to the 300mL level.
3. Add 50mL of dialyzed FBS to the media in the filter unit.

4. Add 5mL of the stable glutamine to the filter unit.
5. Add 5mL of MEM Non-essential amino acids to the filter unit.
6. Add 1mL each of arginine, lysine, and proline to the filter unit.
7. Filter the rest of the media into the bottom bottle.
8. The media is now ready and can be stored at +4C until use.

Notes

The final concentrations of lysine, arginine, and proline in the prepared culture medium are 146mg/L, 84mg/L, and 200mg/L, respectively.