Caco-2 Cell Culture Protocol
From Ainslie Lab @ UNC ainslielab.web.unc.edu

Date: 6/11/07

Media Preparation

Supplies:
Eagle's Minimum Essential Medium (MEM)
450 mL Bottle with:
- Earle's Balanced Salt Solution (BSS)
- 2 mM L-glutamine
- 0.1 mM nonessential amino acids
- 1.5 g/L sodium bicarbonate

1.0 mM sodium pyruvate (for non-ATCC MEM)
(1) – 5 mL aliquot kept in freezer
20% Fetal Bovine Serum (FBS)
(2) – 50 mL aliquots kept in freezer
1% Penicillin-Streptomycin (pen/strep) antibiotic solution
(1) – 5 mL aliquot kept in freezer
0.2 or 0.45 micron media filter with sterile filter and 500 mL bottle

Method:
1. Place MEM and supplements into 37 degrees Celsius water bath. Warm to temperature, approximately 30 minutes.
2. In laminar flow hood, assemble media filter and attach to house vacuum line.
3. Open line to start vacuum.
4. Add media to basin superior to filter. Add warmed supplements. Once all media and supplements are filtered, cap bottle and dispose of filter unit and centrifuge tubes used for aliquots in biohazard trash.
5. Label media bottle "MEM", "20% FBS", "1% P/S", "1% Na Pyruvate", date and initials.
6. Store media in refrigerator until time of use.

Cell Maintenance (Feeding Cells)

Supplies:
Modified Eagle's Minimum Essential Medium (MEM)
- Earle's Balanced Salt Solution (BSS)
- 2 mM L-glutamine
- 0.1 mM nonessential amino acids
- 1.5 g/L sodium bicarbonate
- 1.0 mM sodium pyruvate
- 20% Fetal Bovine Serum (FBS)
- 1% Penicillin-Streptomycin (pen/strep) antibiotic solution

Method: (to be done approximately every 2 days)
1. Heat media to 37 degrees Celsius in water bath.
2. In laminar flow hood, aspirate media with glass Pasteur pipet connected to house vacuum line.
3. Add 15 mL of media.
4. Tightly cap and return to 37 degrees Celsius incubator.

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Passing Cells

Supplies:
Modified Eagle's Minimum Essential Medium (MEM)

- Earle's Balanced Salt Solution (BSS)
- 2 mM L-glutamine
- 0.1 mM nonessential amino acids
- 1.5 g/L sodium bicarbonate
- 1.0 mM sodium pyruvate
- 20% Fetal Bovine Serum (FBS)
- 1% Penicillin-Streptomycin (pen/strep) antibiotic solution

0.25% (w/v) Trypsin – 0.03% (w/v) EDTA
(1) – 10 mL aliquot kept in freezer
Phosphate Buffer Solution (PBS) – optional
Kept in refrigerator in cell culture lab (~450 mL)
15 mL sterile centrifuge tube
75 cm^2 sterile tissue culture flasks

Method:

1. Heat trypsin and media to 37 degrees Celsius in water bath.
2. In laminar flow hood, aspirate media with glass Pasteur pipet connected to house vacuum line.
3. Add 5 mL phosphate buffer solution (PBS) or 0.25% (w/v) Trypsin – 0.03% (w/v) EDTA to remove traces of FBS and residual Ca^{2+} that will deactivate EDTA. Immediately aspirate off solution.
4. Add 3 mL 0.25% (w/v) Trypsin – 0.03% (w/v) EDTA
5. Incubate for ~5 minutes at 37^{o}C.
6. When cells have detached, add 7 mL media to quench trypsin. Reduce clumping by forcefully pipetting mixture against side of flask 4-5 times.
7. Pipette total volume into 15 mL centrifuge tube. Centrifuge for 10 minutes at 1.3 x 1000 rpm.
8. Remove supernatant.
9. Vigorously resuspend Caco-2 cell pellet with 4/6/10 mL media (for 1:4, 1:6, 1:10, respectively).
10. Add 14 mL to each new 75 cm^2 flask.
11. Add 1 mL of Caco-2 cells to each flask.
12. Label and incubate at 37 degrees Celsius. Feed cells by changing media approximately once every two days.