SOP: **Propagation of Caco-2**
Date modified: **9/5/2008**
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Ordering Information

Caco-2 can be ordered from ATCC as a frozen ampoule.

Name:	Caco-2, colorectal adenocarcinoma
ATCC #:	HTB-37

Notes:
This is an adherent cell line.

Materials List

1. MEM with 2mM L-glutamine (Cellgro Cat# 10-010-CM)
2. Fetal Bovine Serum (Cellgro Cat# 35-016-CV)
3. Sodium Bicarbonate (Cellgro Cat# 25-035-CI)
4. Sodium Pyruvate (Cellgro Cat# 25-000-CI)
5. Non-essential amino acids (Cellgro Cat# 25-025-CI)
6. T75 & T225 culture flasks
7. Graduated pipets (1, 5, 25mL)
8. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
9. Hemocytometer
10. Micropipet w/ P20 tips
11. Microscope

Growth Medium for Caco-2

MEM with 2mM L-glutamine
Non-essential amino acids
Sodium Pyruvate 1mM
20% FBS
Pen-Strep (1X)
Sodium Bicarbonate 1.5g/L

Procedure

A. Receipt of frozen cells and starting cell cultures.

1) Immediately place frozen cells in liquid nitrogen storage incubator.
2) Quickly thaw ampoule in 37^{o}C water bath.
3) Transfer thawed cells to a T75 flask with 40mLs of warm growth media.
4) Allow cells to recover over night in 37^{o}C, 5% CO_2 humidified incubator.
5) Pour off medium the next day, replace with fresh medium and return to incubator.

B. Sub-culture

1) Propagate cells until density reaches ~80% confluence (8 X 10^4 – 10^5 cells/cm^2).
2) Decant medium.
3) Wash cells with warm 1X PBS.

4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.
5) Immediately remove cells and pellet at 500 xg for 5 minutes ($4^{o}C$)
6) Wash cells 2X with 1X PBS.
7) Gently re-suspend cell pellet in warm medium.
8) Perform 1:4 to 1:8 cell split as needed. Seed at a density of 10^{4}cells/cm^{2}.
9) Record each subculture event as a passage.

C. Maintenance

1) Change media the day after seeding and 1- 2 times per week.
   Use ~50mLs of medium per T225 flask.

D. Differentiation

1) Upon reaching the desired cell number, cells are grown to confluence. Cells are not harvested until 2 days after confluence to ensure complete differentiation.

E. Harvest

2) Do not use cells that have been passed more than 8 times.
3) Remove cells from flasks according to protocol described above under 'subculturing'.
4) Let cells grow 48 hours past confluence.
5) Examine viability using trypan blue staining (SOP).