SOP: **Propagation of HeLa S3**
Date modified: **10/29/2008**
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Ordering Information

HeLa S3 may be ordered from ATCC as a frozen ampoule.

Name: HeLa S3, cervical carcinoma
ATCC #: **CCL-2.2**

Notes:
This is an adherent cell line. We use DMEM in place of ATCC recommended F-12K medium.

Materials List

1. DMEM with 2mM L-glutamine (cellgro Cat# 10-013-CM)
2. Fetal Bovine Serum (cellgro Cat# 35-016-CV)
3. T75 & T225 culture flasks
4. Graduated pipets (1, 5, 25mL)
5. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
6. Hemocytometer
7. Micropipet w/ P20 tips
8. Microscope

Growth Media for HeLa S3

DMEM with 2mM L-glutamine
10% FBS
1x Pen-Strep

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 37C water bath
3) Transfer thawed cells to a T75 flask with 40ml 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 70-80% confluence.
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 3 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
9) Record each subculture event as a passage

C. Maintenance

1) Change media the day after seeding and every 72 hours thereafter.
   Use 50ml of media per T225

D. Harvest

1) Do not use cells that have been passed more than 25 times
2) Remove cells from flasks according to protocol described above under 'sub-culturing'
3) Examine viability using trypan blue staining.