

SOP: Propagation of HeLa S3
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Modified by: J. Goldy/M. Dorschner

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°C, 5% CO₂ 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°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.