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

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

Name: NTERA-2, testis-malignant pluripotent embryonal carcinoma
ATCC #: CRL-1973

Notes:
This is an adherent cell line. Cells must be scraped for subculturing and harvesting.

Materials List

1. DMEM with 2mM L-glutamine (Cellgro Cat# 10-013-CM)
2. Fetal Bovine Serum (Cellgro Cat# 35-016-CV)
3. Sodium Bicarbonate (Cellgro Cat# 25-035-CI)
4. Cell Scraper (Falcon Cat# 353087)
5. T75 & T225 culture flasks
6. Graduated pipets (1, 5, 25mL)
7. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
8. Hemocytometer
9. Micropipet w/ P20 tips
10. Microscope

Growth Medium for NTERA-2

DMEM with 2mM L-glutamine
10% 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 70-80% confluence.
2) Scrape cells from each flask using cell scraper.
3) Immediately remove cells each flask and pellet at 500 xg for 5 minutes (4^{o}C).
4) Gently re-suspend cell pellet in warm medium.
5) Perform 1:4 to 1:8 cell split as needed. New subcultures should be seeded at $\geq$ 1.5 X 10^7 viable cells per 225 cm^2 flask.

6) Record each subculture event as a passage.

C. Maintenance

1) Change media the day after seeding and every 2-3 days thereafter. Cultures should be maintained at high density. Use ~50mLs of medium per T225 flask.

D. Harvest

1) Do not use cells that have been passed more than 8 times.
2) Remove cells from flasks according to protocol described above under 'subculturing'.
3) Examine viability using trypan blue staining (SOP).