GROWTH CONDITIONS BY TUMOR MODEL

Lines that grow on laminin coated plates:
Astro-111FHTC
ATRT-310FHTC
EPD-210FHTC
GBM-109FHTC
GBM-110FHTC
GBM-511FHTC
PBT-04FHTC
PBT-05FHCT

Lines that grow as spheroids on non-coated plates:
Med-114FHTC
Med-2112FHTC
Med-411FHTC

PRIMARY CELL LINE TISSUE CULTURE

The growth conditions for our primary human pediatric cancer cell lines are a modification of *Lee, et al., (2006) Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. Cancer Cell. 9(5):391-403*.
It has been our experience that the cell lines grow best at densities from 10,000 to 100,000 cells per cm^2, when on laminin coated plates. Cells that form spheroids grow best when maintained at a density of 100,000 to 2s6 cells per ml. Cell lines have a doubling time of approximately 36 to 48 hours when growing well.

MEDIA, SUPPLEMENTS, AND OTHER MATERIAL

NeuroCult NS-A Basal Medium (Human); Stem Cell Technologies Cat# 05750, 450mL.
NeuroCult NS-A Proliferation Supplements – Human (50ml); Stem Cell Technologies Cat # 05753, 50mL.
Epidermal Growth Factor (EGF); AF-100-15, 1mg. Murine EGR, Peprotech Cat # 100-18B, 1 mg, can be substituted for Human EGF.
Fibroblast Growth Factor (FGF); Peprotech Cat # 315-09, 1 mg.
Penicillin – Streptomycin (Penn/Strep)
Laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane 1 mg/mL in Tris buffered NaCl; Sigma-Aldrich Cat # L2020.
Delbecco's Phosphate Buffered Saline without calcium chloride, without magnesium chloride (PBS); Gibco 2017-10.
Accutase; Sigma-Aldrich A6964-100mL.
DNAse1; Stemcell technologies #07900.
Tissue culture treated dishes or flasks.

LAMININ COATING OF PLATES OR FLASKS

Laminin stock solution (1mg/ml) is diluted 1:100 in PBS to make 1x working solution. For example to make 10 mL of working solution add 100 uL of Laminin into 10mL PBS. Completely

cover the bottom of the tissue culture treated plate or flask with 1x Laminin. Place in a 37° C incubator for a minimum of 1 hour.

EGF AND FGF STOCK PREPARATION

Preparation is the same for EGF and FGF.
Dilute 1 mg EGF or FGF in 1 mL PBS (1st Stock solution, 1 mg/mL final concentration).
Dilute 20 µL 1st Stock into 980 microliters of PBS (2nd Stock solution 1000x, 20 µg/mL final concentration).
All stock solutions should be stored at -20° C.
Small volumes of 1000x EGF and FGF may be aliquoted and stored at -20° C.

MEDIA PREPARATION

To one bottle of NeuroCult NS-A Media (450ml), add:

1. One bottle of NeuroCult NS-A Proliferation Supplements – Human (50ml)
2. One aliquot of Pen/Strep (5ml)
3. 500ul of 1000x EGF (20ug/ml)
4. 500ul of 1000x FGF (20ug/ml)
5. Filter sterilize media. Store at 4°.
6. Bring to 37° C before using.

Alternatively if you wish to make smaller amounts of media:
To one bottle of NeuroCult NS-A Media (450 ml) add

1. One bottle of NeuroCult NS-A Proliferation Supplements – Human (50ml)
2. One aliquot of Pen/Strep (5ml)
3. Filter sterilize media.
4. Make 40ml aliquots and store at -20° C.

To use, thaw frozen 40mL aliquot and add:

1. One 40ul aliquot of 1000x EGF
2. One 40ul aliquot of 1000x FGF

ESTABLISHING CULTURES FROM FROZEN CELLS

1. Prepare a 15ml conical tube with 9ml of room temperature PBS.
2. Thaw a cryo-vial of cells rapidly under warm water.
3. Gently pipet the contents of the cryovial into the tube with PBS.
4. Centrifuge (i.e. 300 to 400g for 4 minutes) to pellet cells.
5. Aspirate the supernatant. Note- the cell pellet may be difficult to visualize, aspirate carefully.
6. Resuspend cells in 1ml of complete Neurocult media. Rinse the conical end of the tube with media to collect cells, even if you don't see a distinct pellet.
7. Place cells in a small tissue culture dish (10cm or smaller) or flask (T25).
8. Cells should be allowed to grow into a confluent lawn, or spheroid clusters visible to the eye before dissociating and expanding the culture. Change or add media once or twice a week as necessary.

MAINTAINING ADHERENT CELL CULTURES

Replace media once or twice per week.
When cells become confluent or you wish to pass the cells

1. Remove the media and rinse cells with PBS. Remove PBS.

2. Add room temperature Accutase to completely cover cells and let sit at room temperature or 37^{o} until the cells lift off the plate, typically 3-5 minutes.
3. Collect cells by adding PBS or tissue culture media, transfer to centrifuge tube and spin the cells at 300 to 400 g for 5 minutes
4. Resuspend the cell pellet in 1 mL media and count 20ul of the resulting suspension.
5. Plate cells at approximately 10,000 cells per cm^{2} on freshly laminin coated TC plate or flask.
6. Cells are cryopreserved in media plus 10% DMSO

MAINTAINING SUPENSION CELL CULTURES

When you wish to pass the cells

1. Decant the flask contents into a 50ml conical tube.
2. Pellet cells with a gentle centrifugation at ~300g for 3 minutes
3. Aspirate the supernatant and resuspend the pellet in 1ml of room temperature Accutase. Adding DNAse1 at 0.1mg/ml to the sample can help prevent clumping of cells.
4. Incubate at 37^{o} for 5 -10 minutes.
5. Remove tubes from the incubator and add 9ml of PBS or Media.
6. Centrifuge at 350-400g for 4-5 minutes.
7. Aspirate the supernatant and resuspend cells in 1ml of media.
8. Count 10-20ul of cells to determine the cell concentration and re-plate cells as desired.
9. We have found that for most multi-well plate assay lasting 1 week or less that 0.2e6 cells per ml is appropriate.