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 reviving frozen cell aliquots it is best to plate the cells on a small tissue culture dish (10cm or smaller) or flask (T25). The media should also be changed the next day to remove the DMSO.

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.
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 1-3 hours.

EGF and FGF stock preparation

Preparation is the same for EGF and FGF.
Dilute 1 mg EGF or FGF in 1 mL PBS (1^{st} Stock solution, 1 mg/mL final concentration).
Dilute 20 µL 1^{st} Stock into 980 microliters of PBS (2^{nd} 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

MAINTAINING CELLS

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 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 5 mL media and count 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.