Mayo Clinic Brain Tumor Patient-Derived Xenograft National Resource

Establishing Short-Term Explant Serum-Containing Cultures From Xenograft Lines

Short-term explant cultures can be readily derived from most established xenograft lines, and these cultures can be used for in vitro studies or in preparation for establishing intracranial tumors.

Similar techniques are used for establishing both standard cell cultures using serum-containing media or BTIC (brain tumor initiating cell) cultures using serum-free stem cell media. Although beyond the scope of this protocol document, either culture model can be used for assessing in vitro drug sensitivity using cell proliferation or colony formation assays, performing mechanistic studies regarding drug efficacy, or for studying basic cancer biology in highly relevant tumor models.

When needed in preparation for tumor implantation, these short-term cultures can be transduced with lentiviral vectors for gene knock-down or over-expression studies or for in vivo imaging studies.

The ability to develop short-term cell cultures from xenografts provides tremendous versatility in the use of this xenograft model and can facilitate numerous experimental approaches.

Materials:

- Corning™ Matrigel™ GFR Membrane Matrix (Corning #354230; subsequently referred to as Matrigel through the remainder of the protocol)
- DMEM (Corning #10-013-CV)
- Fetal Bovine Serum (FBS) Premium (Atlanta Biologicals #S11150)
- Penicillin/Streptomycin (Corning #30001CI; 5000 I.U./mL Pen, 5000 ug/mL strep (P/S))
- 500 ml sterile filter (Nalgene: Thermo Scientific #156-4020)
- 2.5% DMEM (2.5% FBS, 1% Pen/Strep, filter-sterilized)
- 10% DMEM (10% FBS, 1% Pen/Strep, filter sterilized)
- 150 mm Tissue Culture Flasks (Corning #430825)
- CO_2 source
- Betadine (Carefusion #29906-016)
- Sterile culture plates
- Sterile scalpels
- 1 cc syringe
- Wet ice

Preparation:

1) Thaw Matrigel aliquots on wet ice.

2) Make up a stock solution for coating flasks by adding 400 microL of Matrigel to 3.6 mL of 2.5% DMEM for each 150 mm flask to be coated. Coat flasks with Matrigel by adding about 4 mL to each one and then tipping the plates in order to coat the entire culture surface.
3) Allow the plates to sit at room temperature on a level surface for about 30 minutes prior to use to allow the Matrigel to properly adhere to the flasks.

Short-term explant culturing:

4) Euthanize a mouse with a 1 to 1.5 cm tumor by CO_2.
5) Swab the tumor area with Betadine, cut out the tumor using a sterile scalpel, separate the tumor from the skin, and mince the tumor sample in a sterile culture plate using a sterile scalpel blade.
6) Add approximately 3 mL of 2.5% DMEM to the plate and continue to disrupt the tumor chunks using a 1 cc syringe.
    - Tip the culture plate on its side and break up the tumor into smaller chunks by pulling it into the syringe and expelling it back into the plate many times. This will allow for better disruption of the tumor and a more even distribution of the cells on the tissue culture flasks.
7) Pull up 1 cc of tumor cells/media and place into each Matrigel-coated flask. If there are still cells/media left over, distribute evenly to each plate. Add an additional 25-35 mL of 2.5% DMEM to the flask.
    - Additional media can be added after tumor disaggregation depending on the number of plates being seeded. Cells seem to grow best using Corning tissue culture plastic.
    - Use of low percentage fetal bovine serum favors growth of glioma cells over murine fibroblasts. No other media supplements are required.
8) Maintain cells in an incubator at 37^oC and 5% CO_2.
9) Check the flasks daily until the cells have adhered to the plates.
    - Depending on the tumor line, this may take 1 to 7 days. Take care to not disturb the cells until they have fully adhered to avoid cell loss.
10) Once cells adhere to the plate, remove the debris from the plate by vigorously shaking the plate and then aspirating the media, debris and nonadherent cells.
    - Do not beat the flasks against your hand as this may dislodge the cells. Similarly, do not wash the flasks with additional media, as this also may dislodge cells. A sterile Pasteur pipette can be used to remove any stubborn debris or tissue chunks.
11) Replace media with 10% DMEM once the cells have adhered to the flask.
12) Monitor the cells until they are at about 80-90% confluent and change media as necessary.
    - Make sure that the cells are well fed. If the media turns yellow, they may not recover from the stress or they may change their response, making it difficult to reproduce experimental results.
    - The ability to passage cultured cells varies significantly for each tumor line. Some tumor lines require passaging at 1:2 or 1:3 dilutions, while others can be cut more aggressively. The dilution ratio must be determined empirically. For those lines obtained from Mayo Clinic, the culture dilution conditions can be provided if necessary. Information about tumor cell line characteristics can be found on the website or by contacting the Sarkaria lab.

- The ability of the cultures to form xenografts tends to decrease with serial in vitro passaging, so only use cells that have been maintained in culture for less than 30 days.

13) When re-plating the cells for in vitro assays, it is not necessary to use Matrigel-coated plates or flasks.