

Mayo Clinic Brain Tumor Patient-Derived Xenograft National Resource

Flank Tumor Implantation

Large numbers of animals can be implanted with tumor in the flank or intracranially in a single session. Several different methods can be used for generating a uniform population of cells for implantation, including direct isolation of a single cell suspension from flank tumors or short-term explant cultures of cells.

While direct isolation of a single cell suspension eliminates the use of cell culture and may have some theoretical advantages, the PDX National Resource at Mayo Clinic has used short-term explant cultures for establishing our large-scale tumor studies.

Short-term explant cultures can provide a relatively pure population of tumor cells with reproducible growth characteristics. Moreover, when implanting a large number of mice, this technique allows cells to be trypsinized throughout the day so that isolated tumor cells are not kept on ice for longer than three to four hours. This method ensures optimal viability of cells throughout the implantation procedure.

While the methods described for direct flank-to-flank tumor injection for serial passaging can be scaled up to inject large numbers of animals with flank tumors, we have found that injection of flank tumors using short-term cultured cells provides more reproducible and uniform tumor growth than does direct tumor passaging (unpublished data), which can be important for therapy evaluation experiments.

Flank tumor implantation

Materials:

- Short-term explant cultured tumor cells
- Corning™ Matrigel™ GFR Membrane Matrix (Corning #354230; subsequently referred to as Matrigel through the remainder of the protocol)
- Trypsin-EDTA (Corning #25-052-CI; 0.05% trypsin/0.53 mM EDTA in HBSS)
- Complete DMEM (10% FBS and 1% P/S)
 - DMEM (Corning #10-013-CV)
 - Penicillin/Streptomycin (Corning #30001CI; 5000 I.U./mL Pen, 5000 ug/mL strep (P/S))
 - Fetal Bovine Serum (FBS) Premium (Atlanta Biologicals #S11150)
- 15 mL and 50 mL Conical Tubes
- Sterile phosphate-buffered saline (PBS: Corning #21040CV)
- 1½ inch 18-gauge hypodermic needles
- 1 cc syringes
- Isoflurane (Novaplus: Piramal Enterprises Ltd NDC# 66794-019-10)
- Centrifuge
- Hemacytometer

- Trypan Blue (0.4%: Corning #25-900-CI)
- Wet ice

Preparation:

- 1) Determine the number of flasks required for the study based on the number of cells that are required per animal and the number of animals in the study. Make sure enough cells are available to inject all the animals (most of the Mayo Clinic xenograft lines yield anywhere from 15 to 25 million cells per flask at 80% confluence).
 - Each animal is implanted with 2E6-5E6 cells using short-term explant cultures established as described in a separate protocol.
- 2) Aspirate off the media, rinse cells with PBS and trypsinize the cells with an ample amount of trypsin. A 150 mm flask will require 5-10 mL of trypsin. Flasks are incubated at room temperature for 3 to 10 minutes. If cells are not releasing from the plastic, the flasks can be incubated at 37°C with careful monitoring of cell lifting. Once cells begin rounding up and can be dislodged from the plate after striking against your palm, proceed to the next step.
- 3) Add an adequate volume of Complete DMEM (10 to 12 ml) to inactivate the trypsin, transfer the cells and media to a conical tube, and centrifuge the cells at 320 RCF for three minutes. Re-suspend the cells in 10 to 20 ml of PBS. Remove a small aliquot of cells for counting and spin the remaining cells at 320 RCF for three minutes. Count the cells using a hemacytometer and calculate the volume of PBS required for a cell concentration of 2E6-5E6 cells per 100 microL.
- 4) Aspirate off the PBS from the cells, making sure not to disturb the cell pellet, and resuspend cells in the appropriate volume of PBS. Place cells on wet ice and pre-chill the syringes.
- 5) Using a 1cc syringe and an 18-gauge needle, draw up 100 microL of cells and 100 microL of Matrigel. Mix the Matrigel and cells in the syringe and remove the air from the syringe.
 - Using Matrigel should provide close to a 99% take rate.
 - Keep your syringes on wet ice to prevent the Matrigel from solidifying prior to injection.
- 6) Anesthetize the mice with isoflurane and inject tumor cells subcutaneously as described below.

Anesthetize the mice:

- 7) Mice are anesthetized with isoflurane in a plastic desiccator. Place the desiccator into an externally vented fume hood. If a hood is not available, the biosafety department should test that laboratory personnel are not being exposed to excessive isoflurane fumes using this method.
- 8) Place a paper towel in the bottom of the desiccator and add 1-2 mL of anesthetic to the towel.
 - Add additional anesthetic as needed to maintain the required effect.
- 9) Place an individual mouse in the desiccator.
- 10) Once the mouse is unconscious and not moving, remove it from the desiccator and mark the ear using an ear punch or other method of animal identification. Because the procedure is quite quick, we typically do not confirm depth of anesthesia and we do not warm the animals during anesthesia. We do observe the mice and if they are unconscious for more than 5 minutes, we will typically group them with other mice to maintain their body temperature.

Inject tumor sample into mouse:

- 11) Swab the back of the mouse with Betadine or rubbing alcohol over the injection site.
Although the operator should wear sterile gown, gloves and mask (typical garb for handling nude mice), an aseptic field with a sterile drape is not necessary.
- 12) Inject all 200 microL of the tumor/Matrigel mixture into the flank of the mouse.
 - The injection site is typically on the posterior/lateral aspect of the lower rib cage. Insert the needle through the skin into the subcutaneous space to inject. You should lift up the skin with your needle prior to injecting to ensure that you are not in the muscle. The needle should be inserted approximately 5 mm beyond the end of the needle bevel.
- 13) While removing the syringe, pinch the injection site for 15 to 30 seconds so that the tumor/Matrigel mixture does not leak out of the injection site.
- 14) Place the animal back in its cage and repeat the process until all animals are injected. Label the cage with the appropriate xenograft number and record the appropriate information in a laboratory book or computer file.
- 15) Observe the mice two to three times a week for tumor development and growth. Depending on the tumor line, detectable tumors that are growing may take 1 to 12 weeks to appear.