[**Mayo Clinic Brain Tumor Patient-Derived Xenograft (PDX) National Resource**](https://www.mayo.edu/research/labs/translational-neuro-oncology/mayo-clinic-brain-tumor-patient-derived-xenograft-national-resource/overview)

**Fresh PDX Cell Culture Instructions**

**Updated Jan. 2, 2023**

**A few important notes:**

* These lines will not behave like established cell lines. Because of the cellular heterogeneity of these PDX lines, each line will have its own unique growth characteristics, and these characteristics may vary from batch to batch.
* The ability to expand these lines is minimal and varies from line-to-line. See the [GBM PDX characteristics database](https://www.mayo.edu/research/documents/sarkaria-gbm-pdx-lines/doc-20503362) for more information regarding cell culture attributes. Ideally, cells should be used fresh or within the first few passages.
* Transduction with lentiviral vectors is possible in these cell lines but may vary by line.
* Whether FBS or stem cultures, similar techniques are used to maintain these PDX lines although some different materials will be needed.
* Each PDX line provided will have a unique lineage, which is highlighted and explained below.
  + Example: 12, 16, 14, 10
    - 12: The GBM PDX line. Came from the 12th patient tumor xenografted.
    - 16: Mouse number with the GBM flank tumor.
    - 14: Previous mouse flank number. #14 flank tumor was passaged to #16 flank.
    - 10: Tumor generation. Number of times the tumor was passed from mouse-to-mouse.
    - VF: Virus-Free. At one point, this line was cleared of the LDEV mouse virus.
      * We no longer test for this as the virus does not affect our studies.
    - G: Our abbreviation for glioblastoma or GBM.
* Users are responsible for confirming the presence of desirable PDX features upon receipt.

**Materials for STEM CELL CULTURES:**

* Stem cell media (StemPro NSC SFM kit: ThermoFisher Scientific #A1050901).
  + To make 500ml, use the following kit components plus L-glutamine and Pen-Strep solution as follows and filter sterilize:
    - KnockOut DMEM/F-12 Basal Media - 500ml
    - StemPro NSC SFM Supplement - 10ml
    - FGF Basic Recombinant Human - 10ug
    - EGF Recombinant Human - 10ug
    - Reagents not included in the kit:
      * L-glutamine (Corning #25005CI) 10ml of 200mM solution
      * Penicillin/Streptomycin (Corning #30001CI; 5000 I.U./mL Pen, 5000 ug/mL strep) 5ml
* 500 ml sterile filter (Nalgene: Thermo Scientific #156-4020).
* Optional: Laminin (Sigma #L2020-1MG) if adherent cultures are needed after passaging.

**Materials for FBS CELL CULTURES:**

* DMEM media (Corning #10-013-CV)
  + To make 500ml, supplement the DMEM as follows and filter sterilize:
    - Fetal Bovine Serum (FBS) Premium (Atlanta Biologicals #S11150) – 50ml
      * [Final] = 10% FBS
    - Penicillin/Streptomycin (Corning #30001CI; 5000 I.U./mL Pen, 5000 ug/mL strep) – 5ml
      * [Final] = 1% P/S
* 500 ml sterile filter (Nalgene: Thermo Scientific #156-4020)

**Care upon receipt:**

1. Open package and carefully remove flasks. Check adherence and confluency under a microscope.

* If adherent, gently aspirate media and replace with the appropriate fresh media.
  + 1. 30 mL minimum per T150 flask. 15 ml minimum per T75 flask.

1. Return to 37°C incubator.
2. Monitor the cells until they are at about 80-90% confluent and change media as necessary. At this point, cells are ready for experimental use.

* 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.
  + Stem media turns yellow more easily than FBS cultures so keep a close eye on all stem cultures.
* Ideally, cells should be used within two weeks of harvest date on the flask.
* If expansion is required, split cells 1:2 and up to 1:5 using trypsin or TrypLE. Change media at least twice per week and many lines will only need to be passaged once per week (passage when ~80% confluent). We do not recommend long-term passaging as it may change cell behavior, render cells non-tumorigenic, and potentially cause genetic drift.
* If cryopreservation of the cells is warranted, use the following cryopreservation medias:
  + Stem cell cryopreservation media: Full stem cell media as shown above (135ml) + 10% DMSO (15ml). Filter sterilize.
  + FBS cell cryopreservation media: Full DMEM/FBS/Pen/Strep media as shown above (85ml) + 33% FBS (50ml) and 10% DMSO (15ml). Filter sterilize.

1. Process as you normally would for plating.

* When re-plating stem cell cultures for in vitro assays, it is necessary to use laminin-coated plates (1ul/cm2 in a few ml of stem media per flask. Incubate at room temperature for 1h before adding cells and more media) if you want adherent cultures. If you do not use a coating, some lines will form neurospheres.