[**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)

**In Vitro Culturing and Experimentation Using Short-Term PDX Explant Cultures**

**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.
* Short-term explant cultures can be readily derived from most established xenograft lines, and these cultures can be used for in vitro studies or establishing tumors in immunocompromised mice.
* For instructions on caring for our PDX short-term explant cultures, please refer to our culture instructions.
	+ The health of your cultures is extremely important for downstream applications. Routinely monitor and change media as needed.
	+ 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 within the first few passages.
* Each PDX line provided will have a unique lineage, which is highlighted and explained below. You should commit the lineage(s) you receive from us to your records somewhere should there be a need to refer back to this in the future.
	+ 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
		- RG: This line was restarted from cryo-preserved tissue.
			* We no longer record as most lines have been restarted.

**Table 1. Examples of GBM PDX Cell Proliferation Rates**

Proliferation rates were determined using the Cell Titer GLO Viability assay (Promega) in FBS cell cultures on Day 1 and Day 7 after plating. Cells were plated at 2000 per well on Day 0. Two established cell lines (U251 and U87) were provided as a comparator.

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| **GBM Cell Lines** | **Fold Proliferation** |
| GBM6 | 3.5 |
| GBM10 | 2.2 |
| GBM14 | 4.4-6.2 |
| GBM26\* | 0.38 |
| GBM43 | 4.9-5.3 |
| GBM44 | 3.4 |
| GBM108 | 3.7-4.8 |
| U251 | 7-10 |
| U87 | 7-10 |

\*As cell number went down over time, FBS cell cultures from this PDX line have limited experimental use in vitro.

**Table 2. Cell number recommendations for in vitro assays**

This list encompasses lines in which we have the most experience. Many of these numbers will depend on the timepoint of interest. The shorter the timepoint, the more cells that may need to be plated as proliferation time will be shorter. The cell numbers used for the Cell Titer GLO and CyQuant assays would be specific to a 5-7 day assay readout. All of the other assay cell numbers would be appropriate for 24-72 hour assays. The established cell line U251 was provided as a comparator.

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|  | **Assays** |
| **GBM Cell Line** | **Cell Titer GLO Viability assay (96-well plate)** | **CyQuant Proliferation assay****(96-well plate)** | **Flow Cytometry for Cell Cycle****(10 cm dish)** | **Immuno-fluorescence (20x20mm coverslip, 6-well plate)** | **Western blotting****(10 cm dish)** |
| GBM6 | 2000-5000 (best in stem) | 2000-5000 | 2 million |  | 2-3 million |
| GBM10 | 2000-5000 (best in FBS) | 2000-5000 | 2 million |  | 2-3 million |
| GBM12 | 500 (stem only) | not advised | 2 million | 500,000\* | 2-3 million |
| GBM39 | 2000-5000 (best in FBS) | 2000-5000 | 2 million |  | 2-3 million |
| GBM43 | 2000-5000 (best in FBS) | 2000-5000 | 2 million |  | 2-3 million |
| GBM44 | 2000-5000 | 2000-5000 | 2 million |  | 2-3 million |
| GBM108 | 2000-5000 | 2000-5000 | 2 million |  | 2-3 million |
| U251 | 1000 | 1000 | 500,000 | 200,000 | 2 million |

\*G12 cells are plated in stem cell media on laminin-coated coverslips for immunofluorescence

**Materials for STEM CELL CULTURES:**

* Stem cell media (StemPro NSC SFM kit: ThermoFisher Scientific #A1050901; subsequently referred to as SCM).
	+ 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
* Laminin (Sigma #L2020-1MG) for coating vessels

**Materials for FBS CELL CULTURES:**

* DMEM medias (Corning #10-013-CV): (1) with FBS supplementation at 2.5% **and** (1) with FBS supplementation at 10%; subsequently referred to as 2.5% and 10% DMEM.
	+ To make 500ml, supplement the DMEM as follows and filter sterilize:
		- Fetal Bovine Serum (FBS) Premium (Atlanta Biologicals #S11150)
			* 50ml = [Final FBS] 10%
			* 12.5ml = [Final FBS] 2.5%
		- Penicillin/Streptomycin (Corning #30001CI; 5000 I.U./mL Pen, 5000 ug/mL strep) – 5ml
			* [Final] = 1% P/S
* 500 ml sterile filters (Nalgene: Thermo Scientific #156-4020)
* Corning™ Matrigel™ GFR Membrane Matrix (Corning #354230; subsequently referred to as Matrigel through the remainder of the protocol) for coating vessels

**Harvest**

1. Cultures taken directly from the flank tumor (refer to protocol on Cell Harvest from Flank Tumor) will initially contain tumor fragments and mouse cells. Maintain these cultures in an incubator at 37°C and 5% CO2.
2. Check the flasks daily until the cells have adhered to the plates.
	* Depending on the tumor line, this may take between 1-7 days. Take care to not disturb the cells until they have fully adhered to avoid cell loss.
3. Once cells adhere to the plate, remove the debris from the plate by vigorously shaking the plate and then aspirating the media, debris, and non-adherent cells.
	* To avoid dislodging cells, refrain from (i) beating the flasks against your hand and (ii) washing with additional media. A sterile Pasteur pipette can be used to remove any stubborn debris or tissue chunks.
4. Replace media with 10% DMEM (FBS culture) or SCM (stem cell culture) once the cells become adherent.
5. Monitor the cells and change media as necessary until they are approx. 80-90% confluent. At this point, they 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.
	* The Sarkaria lab routinely uses cultured cells for in vivo tumor initiation or in vitro experiments from the initial culture. Use cells as soon as possible after receipt. Cells should be maintained in culture for (preferably) <14 days and no greater than 30 days.
	* 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.
6. When re-plating the cells for in vitro assays, it is not necessary to use coated vessels. For most lines, cells will grow in non-adherent spheres in Stem Cell Media without a laminin coating. If adherent stem-like cells are warranted, the flask must first be coated with laminin.
	* Coating instructions: Thaw Laminin at room temperature, add 1ul per cm2 of Laminin in media (150ul laminin in 4 mL for each 150 mm flask of stem cell media, or 75ul in a T75 flask to be coated.) Allow the plates to sit at room temperature on a level surface for 1-2 hours prior to use to allow the Laminin to properly adhere to the plates.