**Mayo Clinic Brain Tumor Patient-Derived Xenograft (PDX) National Resource**

**Instructions – Use of TMZ Resistant Sublines**

**Last updated Oct. 30, 2023**

**A few important notes:**

* Users are responsible for confirming the presence of desirable PDX features upon receipt.
* 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.
* We do not recommend expansion of these cell lines in vitro. Ideally, cells should be used within two weeks of harvest, with few exceptions.
* Transduction with lentiviral vectors is possible in these cell lines but may vary by line and will almost always be more efficient in fresh cell cultures.
* Whether FBS or stem cultures, similar techniques are used to maintain these PDX lines, although some different materials will be needed.

**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 wanting adherent cultures

**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 remove cryovial(s). Store at -80°C if using immediately. Liquid nitrogen must be used for long-term storage.
* If you want stem cultures to grow adherently, you need to prepare coated vessels ahead of time. Instructions are shown below. We do not generally coat vessels when we thaw FBS cultures.
	+ Coating instructions: Thaw Laminin at room temperature, add 1ul per cm2 of Laminin to 3.6 mL of stem cell media for each 150 mm flask to be coated. Allow the plates to sit at room temperature on a level surface for approximately 2 hours prior to use to allow the Laminin to properly adhere to the plates.
1. Pull cryovial(s) from storage and place on dry ice. Bring to your cell culture area.
2. Place the frozen cryovial(s) in a 37°C water bath just until thawed.
3. In the meantime, fill your tissue culture vessel with the appropriate amount of fresh media.
4. Transfer total volume of thawed cells to tissue culture vessel and place in 37°C incubator at 5% CO2.
5. Monitor cultures daily and change media once adhered.

**Important considerations for experiments:**

* Users should review cell line data on our website to see what media conditions their desired lines grow best in. This should dictate the conditions of your experiment as TMZ sensitivity is highly influenced by cell proliferation rates. Unhappy and/or nonproliferative cells = limited/no TMZ response. You need to get through at least two rounds of replication before you will see a TMZ effect, so use your cell line proliferation rates to identify the necessary timepoints for your work.
* TMZ is light sensitive and has a short half-life in aqueous solution. We use in a darkened hood and add to cells immediately after the stock is added to media.

**General recommendations**

Note: Optimization should still be done upon receipt with your specific approaches.

* + Seeding density: 1,000-4,000 cells/well (96w format), 200,000-1,000,000 cells/well (6w format)
	+ TMZ dose ranges: 3-300 µM (FBS conditions), 2-10 µM (stem)
	+ Timepoint: 5-day minimum (see bullet #1 above)
* **Special notes for GBM12:**
	+ This line will not proliferate in FBS-containing media. As a result, we recommend using stem conditions and neurosphere formation assays to gauge sensitivity.
		- GBM12 cells should be plated at 500 cells per well (96-well plate) in a neurosphere formation assay and treated the next day. Use inner 60w (200µl/well) and place PBS around the plate edges.
		- GBM12 parental cells should be treated with a 0, 2, 4, 6, 8, and 10uM dose response while G12 TMZ-resistant cells can be treated up to 300uM (0, 3, 10, 30, 100, 300uM).
		- Neurospheres are counted after 14 days.
		- Descriptions of this assay can be found in some of our publications. See papers by Gaspar Kitange or Shiv Gupta.