Human Glioblastoma Cell Isolation and Protocol

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Reagents:

- Enzymatic dissociation solution (EDS). EDS is 0.025% Trypsin (Gibco Life Technologies, #253-054), 0.5x HBSS (Cellgro, #21-021-CV) and 500 units/ ml each of Collagenase (44,000 units/ ml, Gibco Life Technologies, #17104-019) and Hyaluronidase (Sigma Aldrich, H3506). I.e. a 1:1 solution of 0.05% Trypsin and 1x HBSS supplemented with Collagenase and Hyaluronidase.

- Cell strainer, 100 µm Nylon (BD Falcon #352360, or similar)

- Serum free Neurobasal medium (Gibco Life Technologies, catalog #21103-049); supplemented with N-2 at 1x final concentration (Gibco Life Technologies, catalog #17502-046; 100X), B-27 at 1x final concentration (Gibco Life Technologies, catalog #17504-044; 50X), EGF (50 ng/ml) (#8916LF), bFGF (25 ng/ml) (#8910LF, both Cell Signaling Technology, Beverley MA), and Penicillin/Streptomycin (10 U/ml), (Gibco Life Technologies, catalog #30-009-c3).The fully supplemented material is referred to as “NeuroComplete” media.

- Accutase in DPBS without Ca++ or Mg++; cat. (Innovative Cell Technologies, Inc., catalog. #AT-104, accutase.com).

- Bambanker serum free freezing medium (Wako Pure Chemical Industries, Ltd., catalog . #302-14681)

- Lympholyte-M (Cedarlane Labs., #CL5030)

Protocol:

1) Glioblastoma tumors are received as cooled, fresh PDX material recently harvested from a host mouse. Upon examination, the ideal xenograft tumor is free of central necrosis, between the size of 1,000 to 1,500 mm^3 (1 to 1.5 g), and it should not have any abnormalities.

2) Small tumor pieces are removed and stored in 0.5 ml Bambanker freezing media overnight at -80^oC before being transferred to liquid N_2 for long term storage.

3) The remaining tumor material is diced finely with sterile razor blades or scalpels until no large chunks are present.

4) The finely diced tumor puree is transferred to a 15ml tube with ~1-2ml of EDS per 1 g of tumor material.

5) Tumor material is digested at 37°C in an orbital shaker (or by regular manual agitation to prevent settling) for 30 min. If the material is not digested enough it can be returned to 37°C for another 30 min.

7) The crude cell preparations are filtered through a 100 μm nylon cell strainer and the filtered material brought to 10 ml with NeuroComplete media before centrifugation at 800 g for 5 min. Repeat washing step 1-2 times.

8) The washed cellular material is re-suspended in 5 ml of NeuroComplete media and very gently layered onto 5 ml of Lympholyte M before centrifugation at 1300 xg for 20 min ***with the brake off***.

9) Aspirate all but ~1 ml of the supernatant and carefully remove the cells at the interface.

10) Bring the volume up to 10 ml with NeuroComplete media and wash 2-3 times by centrifugation at 800 xg for 5 min.

11) The resulting cellular material is counted with a Nexcelom Bioscience Cellometer™ Auto T4 counter and seeded at the desired density in ultra-low attachment dishes (Corning, #3262) or onto standard tissue culture vessels pre-coated with 10 μg/ml Laminin (Sigma Aldrich) in 1x PBS for 2-3 hrs at 37°C.

12) Cells are then cultured under “normoxic” conditions in a standard 37°C tissue culture. incubator or in a hypoxia chamber (3% O_2, 5% CO_2, 92% N_2) which sometimes facilitates propagation.

13) Remaining cells are re-suspended in Bambanker media and frozen at -80°C overnight before transfer to liquid N_2.