Culture conditions:

Overview

Primary human GBM cells are cultured in serum-free medium supplemented with EGF and FGF and can be grown as either floating neurospheres[1] or as an adherent monolayer as glioma neural stem (GNS)[2,3] cells on a basement membrane of laminin or matrigel.

Culture medium

The cells have been tested using two serum free culture conditions with equivalent growth patterns.

1. RHB-A® cat#Y40001 http://www.clontech.com

2. StemPro® NSC SFM cat #A1050901 www.thermofisher.com (use as per manufacturer's instructions).

Growth factor supplementation

Epidermal growth factor (EGF) - final concentration (20ng/ml)

Fibroblast growth factor (FGF) - final concentration (10ng/ml)

Basement membrane attachment

For adherent growth purified laminin cat#L2020 http://www.sigmaaldrich.com or Corning® Matrigel® cat#354234 http://www.corning.com (Corning) are diluted 1:100 in cold phosphate buffered saline (PBS) and then filter sterilised. Tissue culture flasks are pre-coated and left to stand in the incubator for a minimum of three hours. Aspirate PBS and seed cells at the desired density.

Cell dissociation

ACCUTASE® cat# A6964-100ML http://www.sigmaaldrich.com

Each bottle contains 100 ml of 1x ACCUTASE enzyme in Dulbecco's PBS (0.2 g/L KCL, 0.2 g/L KH_2PO_4, 8 g/L NaCL, and 1.15 g/L Na_2HPO_4) containing 0.5 mM EDTA·4Na and 3 mg/L Phenol Red.

Use as per manufacturer's instructions.

Cell differentiation

To differentiate cells, remove EGF and FGF from the culture media and replace with 2% foetal bovine serum (FBS). Cells will differentiate within a few hours and adhere to non-matrigel/laminin coated tissue culture flasks and stain positive for neuronal and glial differentiation markers.

References

1. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 1992;255:1707-10.

2. Pollard SM, Yoshikawa K, Clarke ID, et al. Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. Cell Stem Cell 2009;4:568-80.
3. Day BW, Stringer BW, Wilson J, et al. Glioma surgical aspirate: a viable source of tumor tissue for experimental research. Cancers 2013;5:357-371.