REFERENCE GUIDE

NeuroCult™ Reagents for Brain Tumor Stem Cell Research

Culture Methods for Brain Tumor Stem Cells

Multipotent neural stem-like cells or brain tumor stem cells (BTSCs), also known as cancer stem cells (CSCs), have been successfully isolated from a wide range of adult and pediatric central nervous system (CNS) cancers, including gliomas and medulloblastomas.[1] Similar to neural stem cells, these brain tumor stem cells can self-renew, and exhibit high proliferative capacity and multi-lineage differentiation potential in vitro.

Given that prospective identification of BTSCs typically involves isolation of cells followed by in vitro cell culture, the use of appropriate culture methodology is critical.[2-3] The cell culture system must be able to preserve the functional properties and genetic aberrations of the parental tumor. Standard serum-based culture conditions commonly applied to glioma cell lines lead to genetic and epigenetic changes that alter the cellular phenotype.[4] Conversely, when BTSCs are cultured using the serum-free conditions routinely applied to neural stem cells, the genetic and phenotypic properties of the parental tumor are maintained.

Both neurosphere and adherent monolayer culture systems have been shown to support BTSCs. The neurosphere assay may be a clinically relevant functional readout for the study of BTSCs, with research indicating that renewable neurosphere formation in cultured human glioma samples could be a significant predictor of rapid tumor progression and increased risk of patient death.[5-6] The adherent monolayer culture has the advantage of being compatible with a variety of different chemical and genetic screens. Both culture methods have been shown to support their use in the development of patient-derived pre-clinical models, with applications including the testing of personalized glioblastoma therapies.[7]

NeuroCult™ NS-A for Brain Tumor Stem Cells

NeuroCult™ NS-A is the most referenced specialized culture medium for human neural stem cells and brain tumor stem cells. High impact publications demonstrate that NeuroCult™ NS-A Proliferation Kit (Human) supports the isolation and proliferation of brain tumor stem cells from both pediatric and adult tumors, and from a variety of CNS tumor types. Species-specific media and supplements are also available for culturing and differentiating brain tumor stem cells and neural stem cells from mouse and rat CNS cancer models.

Techniques supported by NeuroCult™:

- Dissociation of brain tumor samples into a single cell suspension
- Culture of cells obtained from different types of brain tumors
- Differentiation of brain tumor stem cells into neural and glial lineages
- Passaging/dissociation of tumorspheres

Research supported by NeuroCult™:

- Investigation of the dysregulation of microRNAs and signaling pathways in gliomas[8-10]
- Identification of novel glioma oncogenes[11]
- Study of the genotypic and phenotypic heterogeneity of cells within solid tumors[12,13]
- Assessment of the ability of inhibitors to suppress BTSC proliferation[14-16]
- Validation of animal models of CNS cancers[17]
- Study of lineage plasticity of glioma stem cells[18]
- Development of fluorescent reporter glioblastoma cell lines for in vivo imaging of tumor growth[19]

OPEN ACCESS VIDEO PROTOCOL

Isolation and expansion of human glioblastoma multiforme tumor cells using the neurosphere assay[7]
www.stemcell.com/joveneurosphere

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NeuroCult™ for Brain Tumor Stem Cells

Dissociate Tissue

Dissociate human brain tumor samples:

- Influence of oxygen tension on CD133 phenotype in human glioma cell cultures. Platet N, et al. **Cancer Lett** 258(2): 286-290, 2007

Culture

Culture cells from human glioblastoma multiforme tumors:

- Transcription factors FOXG1 and Groucho/TLE promote glioblastoma growth. Verginelli F et al. **Nat Commun** 4: 2956, 2013
- Intratumoral heterogeneity of receptor tyrosine kinases EGFR and PDGFRA amplification in glioblastoma defines subpopulations with distinct growth factor response. Szerlip NJ, et al. **Proc Natl Acad Sci U S A** 109(8): 3041-3046, 2012
- NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. Fan X, et al. **Stem Cells** 28(1): 5-16, 2012
- YB-1 bridges neural stem cells and brain tumor-initiating cells via its roles in differentiation and cell growth. Fotovati A, et al. **Cancer Res** 71(16): 5569-5578, 2011
- Side population is not necessary or sufficient for a cancer stem cell phenotype in glioblastoma multiforme. Broadley KW, et al. **Stem Cells** 29(3): 452-61, 2011
- PLAGL2 regulates Wnt signaling to impede differentiation in neural stem cells and gliomas. Zheng H, et al. **Cancer Cell** 17(5): 497-509, 2010
- Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. Inda M, et al. **Genes Dev** 24(16): 1731-1745, 2010
- Proteasomal and genetic inactivation of the NF1 tumor suppressor in gliomagenesis. McGillicuddy LT, et al. **Cancer Cell** 16(1): 44-54, 2009
- Gamma-secretase represents a therapeutic target for the treatment of invasive glioma mediated by the p75 neurotrophin receptor. Wang L, et al. **PLoS Biol** 6(11): e289, 2008
- Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Piccirillo SGM, et al. **Nature** 444(7120): 761-765, 2006

Culture cells from human oligodendroglioma tumors:

- Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. Koivunen P, et al. **Nature** 483(7390): 484-488, 2012
- A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. Jenkins RB, et al. **Cancer Res** 66(20): 9852-9861, 2006

Culture cells from human pilocytic astrocytoma tumors:

- Activation of the Hedgehog pathway in pilocytic astrocytomas. Rush SZ, et al. **Neuro Oncol** 12(8): 790-798, 2010

Culture cells from human ependymoma tumors:

- The EphA2 receptor drives self-renewal and tumorigenicity in stem-like tumor-propagating cells from human glioblastomas. Binda E, et al. **Cancer Cell** 22(6): 765-780, 2012
- Effects of epidermal growth factor receptor blockade on ependymoma stem cells in vitro and in orthotopic mouse models. Servidei T, et al. **Int J Cancer**, 2011 (epub ahead of print)

Culture cells obtained from pediatric medulloblastoma tumors:

- Fusion of TTYH1 with the C19MC microRNA cluster drives expression of a brain-specific DNMT3B isoform in the embryonal brain tumor ETMR. Kleinman CL, et al. **Nature Genetics** 46(1): 39-44, 2014.
- Clinical relevance of tumor cells with stem-like properties in pediatric brain tumors. Thirant C, et al. **PloS One** 6(1): e16375, 2011

Differentiate

Differentiate brain tumor stem cells into neural and glial lineages:

- Identification of brain tumour initiating cells using the stem cell marker aldehyde dehydrogenase. Choi SA, et al. Eur J Cancer. 50(1): 137-49, 2014.
- Glioblastoma stem-like cells give rise to tumour endothelium. Wang R, et al. **Nature** 468(7325): 829-833, 2010
- Cyclopamine-mediated hedgehog pathway inhibition depletes stem-like cancer cells in glioblastoma. Bar EE, et al. **Stem Cells** 25(10): 2524-33, 2007.

Passage

Passage/dissociate tumorspheres:

- Oncolytic virus-mediated manipulation of DNA damage responses: synergy with chemotherapy in killing glioblastoma stem cells. Kanai R, et al. **J Natl Cancer Institute** 104(1): 42-55, 2012
- Human glioblastoma-derived cancer stem cells: establishment of invasive glioma models and treatment with oncolytic herpes simplex virus vectors. Wakimoto H, et al. **Cancer Res** 69(8): 3472-3481, 2009
- Girdin maintains the stemness of glioblastoma stem cells. Natsume A, et al. **Oncogene** 31(22): 2715-24, 2012

Advantages of NeuroCult™ NS-A Medium:

SPECIALIZED. Formulated to maintain human neural stem cells and brain tumor stem cells in long-term culture.

VERSATILE. Supports isolation and proliferation of brain tumor stem cells from a variety of tumor types, using either neurosphere or monolayer culture techniques.

CONSISTENT. Conforms to STEMCELL Technologies' stringent quality control standards, including rigorous performance testing using primary cells.

NeuroCult™ for Mouse Brain Tumor Models

Culture cells obtained from mouse models of glioblastoma:

- New strategy for the analysis of phenotypic marker antigens in brain tumor-derived neurospheres in mice and humans. Bleau AM, et al. **Neurosurg Focus** 24(3-4): E28, 2008

Culture cells from mouse models of retinoblastoma:

- Embryonic Retinal Tumors in SV40 T-Ag Transgenic Mice Contain CD133+ Tumor-Initiating Cells. Wadhwa L, et al. **Invest Ophthalmol Vis Sci** 53(7): 3454-3462, 2012

Culture cells from mouse models of neuroblastoma:

- A Mechanism Linking Id2-TGFβ Crosstalk to Reversible Adaptive Plasticity in Neuroblastoma. Chakrabarti L, et al. **PLoS One**. 8(12): e83521, 2013

Culture cells from human-mouse glioma xenograft models:

- Self-renewal does not predict tumor growth potential in mouse models of high-grade glioma. Barrett LE, et al. **Cancer Cell** 21(1): 11-24, 2012

Differentiate brain tumor stem cells from mouse tumor suppressor models:

- Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. Alcantara Llaguno S, et al. **Cancer Cell** 15(1): 45-56, 2009

Culture cells obtained from mouse models of medulloblastoma:

- Targeting placental growth factor/neuropilin 1 pathway inhibits growth and spread of medulloblastoma. Snuderl M, et al. **Cell** 152(5): 1065-1076, 2013
- An animal model of MYC-driven medulloblastoma. Pei Y, et al. **Cancer Cell** 21(2): 155-167, 2012
- Medulloblastoma can be initiated by deletion of Patched in lineage-restricted progenitors or stem cells. Yang Z, et al. **Cancer Cell** 14(2): 135-145, 2008

NeuroCult™ Reagents for Brain Tumor Stem Cell Research

Ordering Information

NeuroCult™ for Human Brain Tumor Stem Cells

PRODUCT	APPLICATION	CATALOG #
NeuroCult™ NS-A Basal Medium (Human)	Basal Medium	05750
NeuroCult™ NS-A Proliferation Kit (Human)*	Proliferation Medium	05751
NeuroCult™-XF Proliferation Medium*	Xeno-Free Proliferation Medium	05761
NeuroCult™ NS-A Differentiation Kit	Differentiation Medium	05752

NeuroCult™ for Rodent Brain Tumor Models

PRODUCT	APPLICATION	CATALOG #
NeuroCult™ Proliferation Kit	Mouse Proliferation Medium	05702
NeuroCult™ NS-A Proliferation Kit (Rat)*	Rat Proliferation Medium	05771
NeuroCult™ Neural Colony-Forming Cell (NCFC) Assay Kit	Colony Assay to Quantify Cells with High Proliferative Potential	05740 (Mouse)
		05742 (Rat)*
NeuroCult™ Differentiation Kit (Mouse)	Mouse Differentiation Medium	05704
NeuroCult™ Differentiation Kit (Rat)	Rat Differentiation Medium	05772
NeuroCult™ Enzymatic Dissociation Kit for Adult CNS Tissue (Mouse and Rat)	Enzymatic Dissociation of CNS Tissue	05715
NeuroCult™ Chemical Dissociation Kit (Mouse)	Chemical Dissociation of Neurospheres	05707

*Requires supplementation with rh EGF (Catalog #02633). When culturing cells obtained from human, rat or adult mouse samples, rh bFGF (Catalog #02634) and Heparin (Catalog #07980) are also required.

Non-Immunological Identification of Brain Tumor Stem Cells

ALDEFLUOR™ (Catalog #01700) can be used to identify, enumerate and isolate viable brain tumor stem cells, based on aldehyde dehydrogenase (ALDH) enzyme activity.[20-24] **www.aldefluor.com**

REFERENCES

Full list of NeuroCult™ brain tumor references
www.stemcell.com/BTSCRefs

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