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DATA SHEET

Human Glioblastoma Cancer Stem Cell (GBM) – T75 Plated Cells

Catalog number: 36104-41-T75

Description: Human Glioblastoma Cancer Stem Cell (GBM) T75 Plated Cells. Also available in T25, T75, T150 and T225 tissue culture flask with plated Cells, shipped at room temperature or in Frozen Vial, shipped in Dry-ice. The Clonal selected Human Glioblastoma Cancer Stem Cell (GBM) was derived from Human Brain Cancer tissue. They were maintained in Celprogen's Human Glioblastoma Cancer Stem Cell (GBM) Complete Growth Medium and sub-cultured every 24 to 48 hours on Human Glioblastoma Cancer Stem Cell (GBM) Extra-cellular Matrix.

Source: Human Brain Cancer tissue

Donors: All donors from which the Cells were derived were pre-screened; donors tested negative for the usual blood donation infectious disease panel (ABO/RH, Hepatitis B Surface Antigen, HIV1 and 2, Syphilis, hepatitis B Core, Human T Lymphocyte Virus 1 and 2, Hepatitis C Virus, Antibody Screen, Nucleic Amplification Test for HIV 1 HCV, West Nile Virus and Antibodies to Trypanosoma cruzi (the agent of Chagas disease).

Storage Conditions: The plated cells are shipped at room temperature. Upon receipt of the plated cells wipe the flask with 70% ethanol and transfer to sterile tissue culture hood. In the tissue culture hood remove the media from the cells and collect in sterile centrifuge tubes, do not throw away the supernatant since during shipping some cells may have detached and will be part of the supernatant, which will need to be centrifuged at 100g for 7 minutes to collect the cell pellet. For the attached cells place 10ml of fresh pre-warmed media and incubate at 37^0C for two hours. After two hours incubation wash the cells with 1X PBS sterile solution, for 2-3 minutes, remove the 1X PBS solution and then Trypsinize. After Trypsinization of the Cells neutralize the Trypsin with equal volume of Human Glioblastoma Cancer Stem Cell Complete Growth Media with Serum and collect the Cell suspension in sterile conical centrifuge tube in the tissue culture hood. Centrifuge the cell suspension at 100g for 7 minutes in centrifuge. Pool the cell pellets from the supernatant cells (detached cells during shipping) and plate cells $5x10^5$ cells per pre- coated flasks with Human Glioblastoma Cancer Stem Cell (GBM) Extra-cellular Matrix for Expansion in Human Glioblastoma Cancer Stem Cell (GBM) Complete Growth Medium.

Positive Markers: BRAF, CD133, P53, CD90, PDFR, O_4, CD44, GFAP, ALDH, Oct4, SSEA ¾, Tumorigenicity (<1000 cells)

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Morphology & Proliferation: Mixed population of cells with approximately 95% attached cells and the other 5.0% in suspension, need to change cell culture media every day after 48 hours of initial cell culture or when the media starts changing color to slight yellow from pink. Fast growing cell culture, change media with Celprogen's Human Glioblastoma Cancer Stem Cell (GBM) Complete Growth Medium with the appropriate Human Glioblastoma Cancer Stem Cell (GBM) Extra-cellular Matrix. Temperature 37^0C in 5% CO_2 humidified incubator.

Sub-culturing of Plated T75 cells:

1. Remove flask and wipe with 70% ethanol. Then transfer to the tissue culture hood. Refer to protocols, flow diagrams and videos for more detail.
2. Transfer the supernatant contents to a sterile centrifuge tubes, this will contain detached cells. Centrifuge at 100g for 7 minutes to obtain cell pellet. Plate cells in a T75 flask pre-coated with ECM.
3. Add pre-warmed Human Glioblastoma Cancer Stem Cell (GBM) Complete Growth Media to flask that has attached cells and incubate for two hours in the incubator. After 2 hours wash the cells with 1X PBS, remove 100% 1X PBS solution.
4. Trypsinize the attached cells with 5ml 1X Trypsin EDTA for 2-3 minutes. At the end of Trypsinization add equal volume of complete growth media with serum. Centrifuge the cells at 100g for 7 minutes to obtain cell pellet.
5. Add the 500ul of cells to T75 flask pre-coated with Human Glioblastoma Cancer Stem Cell (GBM) Extra- cellular Matrix with 10ml of Human Glioblastoma Cancer Stem Cell (GBM) Complete Growth Medium. Depending on the size of the pellet you may utilize a new flask or you may combine the cells to
the flask that had the supernatant cells.
6. Incubate the cells in the T75 flask at 37^0C in a 5% CO_2 humidified incubator. Perform 100% Media Change every 24 to 48 hours.
7. Medium renewal every other day or 2-3 days, sub-culturing ratio: 1:2 or 1:3 depending on the cell density.
8. Refer to protocols, flow diagrams and videos for more detail. http://celprogen.com/tech.htm

Freezing Medium: Available for purchase Cat# M36104-41FM

Trypsin: Available for purchase Cat# T1509-014

IX PBS: Available for Purchase Cat# P1408-013

Storage temperature: Liquid nitrogen vapor phase

Product Orders: Before submitting an order you will be asked to read and accept the terms and conditions of Celprogen's Material Transfer Agreement (MTA).

Permits/Forms: In addition to the MTA mentioned above, other CELPROGEN and/or regulatory permits may be required for the transfer of this CELPROGEN

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material. Anyone purchasing CELPROGEN material is ultimately responsible for obtaining the permits.

Notices & Disclaimers:

***CELPROGEN products are intended for laboratory research purposes only*. They are not intended for use in Humans.** The product, Human Glioblastoma Cancer Stem Cell (GBM), is established and manufactured by CELPROGEN Inc., and is for Research Use Only. This product is not for re- sale or may not be transferred to a third party prior to written request and approval by CELPROGEN Inc.