Human Liver Cancer Stem Cell Culture Data Sheet

**Product name:** Human Liver Cancer Stem Cell Culture

**Catalog number:** 36116-43

**Description:** Frozen Ampule (1.2 x 10^6 cells) of 1 x 10^6 Viable cell upon thawing, shipped with dry-ice. Also available in a T25 or a T75 tissue culture flask with plated cells, shipped at room temperature. Clonal Liver Cancer Stem Cells were derived from Human Liver tissue. They were maintained in Human Liver Cancer Stem Cell Complete Growth Medium and sub-cultured every 24 to 48 hours on Human Liver Cancer Stem Cell Extra-cellular Matrix.

**Source:** Human Liver Cancer tissue

**Donors:** All donors from which the Cells were derived were pre-screened; donors are tested 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:** Liquid nitrogen vapor phase for frozen Ampule of Human Liver Cancer Stem Cell. For plated cells in T75 tissue culture flask, upon receipt of the cells wipe the flask with 70% ethanol and transfer to sterile tissue culture hood. In the tissue culture hood remove the media of the cells and wash the cells with 1X PBS sterile solution (Cat# P1408-013), for 2 -3 minutes, remove the PBS solution and then trypsinize (Cat# T1509-014). After trypsinization of the Cells neutralize the trypsin with equal volume of Human Liver 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. Plate cells 5x10^5 cells per pre-coated flasks with Human Liver Cancer Stem Cell Extra-cellular Matrix for Expansion in Human Liver Cancer Stem Cell Complete Growth Medium.

**Positive markers:** Oct 4: CD 133: Nestin, telomerase, SSEA 3/4 , AP, AFP, CEA

**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 for pink. Fast growing cell culture. Change media with #M36116-43 Human Liver Cancer Stem Cell Culture Complete Growth Medium with the appropriate Human Liver Cancer Stem Cell Extra-cellular Matrix. Temperature 37°C in 5% CO₂ humidified incubator.
Sub-culturing:

1. Thaw the vial with gentle agitation in a 37°C water bath or a dry 37°C shaking incubator. For water bath thawing keep the O-ring out of the water.
2. Remove the thawed vial and wipe with 70% ethanol. Then transfer to the tissue culture hood.
3. Transfer the vial contents to a sterile centrifuge tube, and gently add pre-warmed Human Liver Cancer Stem Cell Growth Media to the centrifuge tube. Use additional Human Liver Cancer Stem Cell Culture Complete Media to rinse the vial and transfer the liquid to the centrifuge tube repeat this once more to ensure you have all the cells transferred to the 15ml centrifuge tube. Centrifuge the cells at 1500 RMP for 5 minutes. Remove the supernatant and re-suspend the cell pellet in 500ul of Human Liver Cancer Stem Cell Culture Complete Growth Medium.
4. Add the 500ul of cells to T75 flask pre-coated with Human Liver Cancer Stem Cell Extra-cellular Matrix with 15ml of Human Liver Cancer Stem Cell Complete Growth Medium.
5. Incubate the cells in the T75 flask in a 37°C in 5% CO₂ humidified incubator. Perform 100% Media Change every 24 to 48 hours.
6. Medium renewal every other or 2-3 days, sub-culturing ratio: 1:3

Trypsin: Available for purchase Cat# T1509-014

1X PBS: Available for purchase Cat# P1408-013

Freezing Medium: Available for purchase Cat# M135501-06

Storage Temperature: Liquid nitrogen vapor phase