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DATA SHEET

Human Embryonic Stem Cell – Frozen Vial

Catalog number: 36101-28

Description: Frozen Ampule (1.2 x 10^6 cells) of 1 x 10^6 viable cells upon thawing, shipped with dry-ice. Also available in T25, T75, T150, and T225 tissue culture flask with plated cells, shipped at room temperature. The Human Embryonic Stem Cell was derived from Human Embryonic tissue. They were maintained in Celprogen's Human Embryonic Stem Cell Complete Growth Medium and sub-cultured every 24 to 48 hours on Human Embryonic Stem Cell Extracellular Matrix.

Source: Human Embryonic tissue

Mycoplasma test: Negative-PCR and mycoplasma agar methods

Sterility: Negative for bacteria, yeast, and mold

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:

Frozen Vial: Liquid nitrogen vapor phase for frozen Ampule of Human Embryonic Stem Cell .

Plated Cells: For plated cells in 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 from the cells and 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 Embryonic 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 Embryonic Stem Cell Extra-cellular Matrix for Expansion in Human Embryonic Stem Cell Complete Growth Medium.

Thawing of Cryovials: It is vital to thaw cells correctly in order to maintain the viability of the culture and enable the culture to recover more quickly. Some cryoprotectants, such as DMSO, are toxic above 4^0C therefore it is essential that cultures are thawed quickly (< 1 minute) and diluted in complete growth media with serum to minimize the toxic effects. Briefly, remove the cryovial containing the frozen cells from dry ice or from liquid nitrogen vapor phase, and immediately place in 37^0C water bath or 37^0C dry oven in a shaker. Quickly thaw the cell (< 1-2 minutes) by gently swirling the vial in the 37^0C water bath or 37^0C dry oven in a shaker, until there is just a small bit of ice left in the vial. Spray the vial with 70% alcohol and transfer the thawed vial in the tissue culture hood. [See protocol for detail]

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Positive Markers: CD 34, CD 133, Nestin, Oct4, SSEA1, SSEA3/4, TRA1-60, TRA1-81, CCR4, CD117, Sox2, AP.

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. This is a fast growing cell culture (with a population doubling time of 24 hours). Change media with Celprogen's Human Embryonic Stem Cell Complete Growth Medium with the appropriate Human Embryonic Stem Cell Extra-cellular Matrix. Temperature 37°C in 5% CO_2 humidified incubator. Population doubling is 80-120 or up to 12 passages when cultured at 60% to 70% confluent in Celprogen culture system. The cells are shipped at passage 2.

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 Embryonic Stem Cell Complete Growth Media to the centrifuge tube. Use additional Human Embryonic Stem Cell Complete Growth 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 100g for 7 minutes. Remove the supernatant and re-suspend the cell pellet in 500ul of Human Embryonic Stem Cell Complete Growth Medium.
4. Add the 500ul of cells to the appropriate size flask pre-coated with Human Embryonic Stem Cell Extra-cellular Matrix with 10ml of Human Embryonic Stem Cell Complete Growth Medium.
5. Incubate the cells in the appropriate size flask at 37°C in a 5% CO_2 humidified incubator. Perform 100% Media Change every 24 to 48 hours.
6. Medium renewal every other day or 2-3 days, sub-culturing ratio: 1:2 or 1:3 depending on the cell density.
7. Refer to protocols, flow diagrams and videos for more detail. http://celprogen.com/tech.htm

Seeding cells from Plated Tissue culture flasks:

For plated cells in 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 from the cells and 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 Embryonic Stem Cell Culture 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 Embryonic Stem Cell Culture Extra-cellular Matrix for Expansion in Human Embryonic Stem Cell Culture Complete Growth Medium.

Freezing Medium: Available for purchase Cat# M36101-28FM

Trypsin: Available for purchase Cat# T1509-014

IX PBS: Available for Purchase Cat# P1408-013

Storage temperature: Liquid nitrogen vapor phase

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

Biosafety Level **1**

Notices & Disclaimers: ***CELPROGEN products are intended for laboratory research purposes only. They are not intended for use in humans.*** The Product, Human Embryonic Stem Cell , 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.