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DATA SHEET

Rat Embryonic Stem Cell Culture – T225 Plated Cells

Catalog number:	55101-28-T225
Description:	Rat Embryonic Stem Cell Culture T225 Plated Cells. Also available in T25, T75, and T150 tissue Stem Cell Culture flask with plated Cells, shipped at room temperature or in Frozen Vial, shipped in Dry-ice. The Embryonic Stem Cell Culture was derived from Rat Embryonic tissue. They were maintained in Celprogen's Rat Embryonic Stem Cell Culture Complete Growth Medium and sub-Stem Cell Cultured every 24 to 48 hours on Rat Embryonic Stem Cell Culture Extra-cellular Matrix.
Source:	Rat Embryonic tissue
Mycoplasma test:	Negative-PCR and mycoplasma agar methods
Sterility:	Negative for bacteria, yeast, and mold
Storage Conditions:	Liquid nitrogen vapor phase for frozen Ampule of Rat Embryonic Stem Cell Culture. For plated cells in tissue Stem Cell Culture flask, upon receipt of the cells wipe the flask with 70% ethanol and transfer to sterile tissue Stem Cell Culture hood. In the tissue Stem Cell 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 Rat Embryonic Stem Cell Culture Complete Growth Media with Serum and collect the Cell suspension in sterile conical centrifuge tube in the tissue Stem Cell Culture hood. Centrifuge the cell suspension at 100g for 7 minutes in centrifuge. Plate cells 5×10^5 cells per pre-coated flasks with Rat Embryonic Stem Cell Culture Extra-cellular Matrix for Expansion in Rat Embryonic Stem Cell Culture Complete Growth Medium.
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 Stem Cell Culture media every day after 48 hours of initial cell Stem Cell Culture or when the media starts changing color to slight yellow from pink. Fast growing cell Stem Cell Culture, change media with Celprogen's Rat Embryonic Stem Cell Culture Complete Growth Medium with the appropriate Rat Embryonic Stem Cell Culture Extra-cellular Matrix. Temperature 37°C in 5% CO ₂ humidified incubator.



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Sub-culturing:

1. Remove flask and wipe with 70% ethanol. Then transfer to the tissue Stem Cell 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 T225 flask pre-coated with ECM.
3. Add pre-warmed Rat Embryonic 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 10ml 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 T225 flask pre-coated with Rat Embryonic Extra-cellular Matrix with 30ml of Rat Embryonic 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 T225 flask at 37°C in a 5% CO₂ 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# M55101-28FM

Trypsin: Available for purchase Cat# T2259-014

1X 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 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, Rat Embryonic Stem Cell Culture, 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.