3914 Del Amo Blvd Suite 901 Torrance, CA 90503 www.celprogen.com



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DATA SHEET

Product name: Rat T Cell -Plated T225

Catalog number: 12124-16-T225

Description:

Rat T Cell T225 Plated Cells. Also available in T25, T75, and T150 tissue culture flasks with plated Cells, shipped at room temperature or Frozen vial shipped in Dry-ice. They weremaintained in Celprogen's Rat T Cell Complete Growth Medium and sub-cultured every 24 to 48 hours on Rat T Cell Extra-cellular Matrix.

Source: Rat Peripheral Blood

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 T Cell . 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 of thecells and wash the cells with 1X PBS sterile solution, for 2-3 minutes, remove the PBS solution and then trypsinize. After trypsinization of the Cells neutralize the t rypsin with equal volume of Rat T 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 Rat T Cell Extra-cellular Matrix for Expansion in Rat T Cell Complete Growth Medium.

Tests Performed:	Microbial: Negative Incorporation of acetylated Low Density Lipoprotein (LDL): Positive
Positive Markers:	CD3, CD4, CD25, CD69, CD154
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 Celprogen's Rat T Cell Complete Growth Medium with the appropriate Rat T Cell Extra-cellular Matrix. Temperature 37^{0} C in 5% CO ₂ humidified incubator.

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Sub-culturing:

- 1. That the vial with gentle agitation in a 37^{0} C water bath or a dry 37^{0} C shaking incubator. For water bath thaving 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 Rat T Cell Growth Media to the centrifuge tube. Use additional Rat T Cell 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 Rat T

1500 RMP for 5 minutes. Remove the supernatant and re-suspend the cell pellet in 500ul of Rat T Cell Complete Growth Medium.

- **4.** Add the 500ul of cells to T225 flask pre-coated with Rat T Cell Extra-cellular Matrix with 15ml of Rat T Cell Complete Growth Medium.
- 5. Incubate the cells in the T225 flask in a 37^{0} 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

Freezing Medium:	Available for purchase Cat# M12124-16FM
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 material. Anyone purchasing CELPROGEN material is ultimately responsible for obtaining the permits.
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