

## DATA SHEET

**Product name:** Rat Osteoblast -Plated T225

**Catalog number:** 12115-07-T225

**Description:**

Rat Osteoblast 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 were maintained in Celprogen's Rat Osteoblast Complete Growth Medium and sub-cultured every 24 to 48 hours on Rat Osteoblast Extra-cellular Matrix.

**Source:** Rat Bone

**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 Osteoblast . 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 the cells 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 trypsin with equal volume of Rat Osteoblast 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  $5 \times 10^5$  cells per pre-coated flasks with Rat Osteoblast Extra-cellular Matrix for Expansion in Rat Osteoblast Complete Growth Medium.

**Tests Performed:** Microbial: Negative  
Incorporation of acetylated Low Density Lipoprotein (LDL): Positive

**Positive Markers:** Troponin, alpha-actin, MEF 2C transcription factor, CD133 & Nestin

**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 Osteoblast Complete Growth Medium with the appropriate Rat Osteoblast Extra-cellular Matrix. Temperature  $37^{\circ}\text{C}$  in 5%  $\text{CO}_2$  humidified incubator.

### **Sub-culturing:**

1. Thaw the vial with gentle agitation in a 37<sup>0</sup>C water bath or a dry 37<sup>0</sup>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 Rat Osteoblast Growth Media to the centrifuge tube. Use additional Rat Osteoblast 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 Osteoblast Complete Growth Medium.
4. Add the 500ul of cells to T225 flask pre-coated with Rat Osteoblast Extra-cellular Matrix with 15ml of Rat Osteoblast Complete Growth Medium.
5. Incubate the cells in the T225 flask in a 37<sup>0</sup>C in 5% CO<sub>2</sub> 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# M12115-07FM

**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.

### **Notices**

**& Disclaimers:** *CELPROGEN products are intended for laboratory research purposes only. They are not intended for use in humans.* This Product Rat Osteoblast 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 third party prior to written request and approval by CELPROGEN Inc.