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# DATA SHEET

## Mouse Retinal Stem Cell – Frozen Vial

**Catalog number:** 66105-32

**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 Mouse Retinal Stem Cell was derived from Mouse Retina Tissue. They were maintained in Celprogen's Mouse Retinal Stem Cell Complete Growth Medium and sub-cultured every 24 to 48 hours on Mouse Retinal Stem Cell Extracellular Matrix.

**Source:** Mouse Retina Tissue

**Mycoplasma test:** Negative-PCR and mycoplasma agar methods

**Sterility:** Negative for bacteria, yeast, and mold

**Storage Conditions:**

**Frozen Vial:** Liquid nitrogen vapor phase for frozen Ampule of Mouse Retinal 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 Mouse Retinal 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 5x$10^5$ cells per pre-coated flasks with Mouse Retinal Stem Cell Extra-cellular Matrix for Expansion in Mouse Retinal Stem Cell Complete Growth Medium.

**Positive Markers:** Sox2, Pax-6, Nestin, CD117, B Tubulin III, GFAP

**Morphology & Proliferation:** Mixed population of cells with approximately 95% attached cells and the other 5.0% in suspension, need to change Stem Cell media every day after 48 hours of initial Stem Cell or when the media starts changing color to slight yellow from pink. Fast growing Stem Cell, change media with Celprogen's Mouse Retinal Stem Cell Complete Growth Medium with the appropriate Mouse Retinal Stem Cell Extra-cellular Matrix. Temperature 37°C in 5% $CO_2$ humidified incubator. Population doubling is 120 or up to 12 passages when cultured at 60% to 70% confluent in Celprogen culture system.

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**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 Mouse Retinal Stem Cell Complete Growth Media to the centrifuge tube. Use additional Mouse Retinal 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 Mouse Retinal Stem Cell Complete Growth Medium.
4. Add the 500ul of cells to T75 flask pre-coated with Mouse Retinal Stem Cell Extra-cellular Matrix with 10ml of Mouse Retinal Stem Cell Complete Growth Medium. Add 500 ul of cells to T25 flask precoated with Mouse Retinal Stem Cell Extracellular Matrix with 5ul of Media.
5. Incubate the cells in the T25 or T75 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 Mouse Retinal 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 Mouse Retinal Culture Extra-cellular Matrix for Expansion in Mouse Retinal Culture Complete Growth Medium.

**Freezing Medium:** Available for purchase Cat# M66105-32FM

**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).

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**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, Mouse Retinal 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.