Rev. 0

Human Pluripotent Stem Cell Derived-Pericytes (HPSC-P)

Catalog #1230

Cell Specification

Pericytes are specialized mural cells that reside along the abluminal surface of capillaries and microvessels, where they interact closely with endothelial cells and contribute to vascular stabilization, vessel maturation, extracellular matrix remodeling, and regulation of vascular permeability [1]. Pericytes play important roles in angiogenesis, blood-brain barrier maintenance, tissue repair, inflammation, and vascular dysfunction associated with disease [2]. Human pluripotent stem cell-derived pericytes provide a valuable *in vitro* model for studying vascular biology, endothelial-pericyte interaction, blood-brain barrier modeling, angiogenesis, fibrosis, and vascular contributions to neurological and systemic diseases [3]. Human Pluripotent Stem Cell-derived Pericytes (HPSC-P) from ScienCell Research Laboratories provide a robust, reliable, and consistent off-the-shelf option for human pericyte research.

HPSC-P are cryopreserved and delivered frozen. Each vial contains > 1×10^6 cells in 1 ml volume. HPSC-P are characterized by immunofluorescence with antibodies specific to pericyte markers platelet-derived growth factor receptor beta (PDGFRβ), neural/glial antigen 2 (NG2), and alpha-smooth muscle actin (α-SMA). HPSC-P are negative for mycoplasma, bacteria, yeast, and fungi. HPSC-P can be cultured under the conditions provided by ScienCell Research Laboratories.

Product Content

Cat. #	# of vials	Product	Quantity	Storage
1230	1	HPSC-P	1 mL	Liquid Nitrogen
5231	1	Pericyte Maintenance Medium (PMM)	100 mL	4°C
5232	1	Pericyte Maintenance Supplement (PMS 50X)	2 mL	-20°C

Recommended Medium

It is recommended to use Pericyte Maintenance Medium (PMM, Cat. #5231) for culturing HPSC-P *in vitro*.

Additional Materials Recommended (Not provided)

Cat. #	Product	Vendor
8248	Bovine Plasma Fibronectin	ScienCell Research Laboratories
5813	CellEase	ScienCell Research Laboratories
0303	DPBS	ScienCell Research Laboratories
1254	ROCK Inhibitor Y-27632	Tocris Bioscience

Product Use
HPSC-P are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage
Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping
Dry ice.

References
[1] Armulik, A., Genové, G., & Betsholtz, C. (2011). Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. Developmental Cell, 21(2), 193–215.
[2] Sweeney, M. D., Ayyadurai, S., & Zlokovic, B. V. (2016). Pericytes of the neurovascular unit: key functions and signaling pathways. Nature Neuroscience, 19(6), 771–783.
[3] Orlova, V. V., et al. (2014). Generation, expansion and functional analysis of endothelial cells and pericytes derived from human pluripotent stem cells. Nature Protocols, 9(6), 1514–1531.

Instructions for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

Note: *Experiments should be well organized before thawing HPSC-P.*

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37°, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. For culturing HPSC-P, we recommend plating the cells on bovine plasma fibronectin-coated culture vessels. Prepare bovine plasma fibronectin-coated culture vessel according to the manufacturer's instructions, and warm to room temperature before using.
2. Prepare complete Pericyte Maintenance Medium (PMM, Cat #5231). Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer Pericyte Maintenance Supplement (PMS 50X) to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.

   Note: We recommend pre-warming the complete medium to room temperature (Not 37°C), prior to use.
3. Prepare a 50 ml conical tube with complete medium. We recommend adding at least 9 mL of the complete medium to the conical tube. Add ROCK inhibitor Y-27632 to a final concentration of 10 μM.

   Note: Applying ROCK inhibitor Y-27632 in the first 24 hours improves the cell viability.
4. Leave the tube in the sterile field and proceed to thaw the cryopreserved cells.
5. Place the frozen vial in a 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field. Carefully remove the cap without touching the interior threads.
6. Gently dispense the contents of the vial into the conical tube containing the complete medium.

   Note: Centrifugation of cells after thawing is not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture.
7. **Gently invert** the tube **1-2 times** to obtain a homogenous cell suspension in the medium. Resuspending neurons by pipetting up and down is NOT recommended.
8. Aspirate coating solution in the prepared culture vessel. Gently aliquot the cell suspension onto prepared culture vessels. We recommend plating the cells at 10,000 cell/cm^2.
9. Replace the lid of the culture plate and gently rock the plate to distribute the cells evenly.

10. Return the culture vessel to the incubator.
11. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells, then every other day thereafter.

Subculturing:

1. Subculture when the culture reaches 90-95% confluency.
2. Prepare bovine plasma fibronectin-coated culture vessels at least 1 hour before.
3. Warm Pericyte Maintenance Medium, CellEase (Cat. #5813) and DPBS (Ca^{++}- and Mg^{++}-free, Cat. #0303) to room temperature. We do not recommend warming reagents and medium in a 37°C water bath prior to use.
4. Rinse the cells with DPBS.
5. Dissociate the pericytes with CellEase protocol.

   Note: We recommend using CellEase which is optimized to minimize cell damage due to over digestion.
6. Gently resuspend cells in Pericyte Maintenance Medium.
7. Count and plate cells in a new culture vessel at desired cell density for assay. A seeding density of 10,000 cells/cm^2 is recommended for expansion.

   Note: We do not recommend cryopreservation of HPSC-P cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee HPSC-P cells cryopreserved by the end user.