Rev. 0

Human Induced Pluripotent Stem Cell-derived Astrocytes (HiPSC-A)

Catalog #1860

Cell Specification

Astrocytes are the major cell type in the mammalian brain. They provide a variety of supportive functions to their partner neurons in the central nervous system (CNS), such as neuronal guidance during development, and nutritional and metabolic support throughout life [1]. Astrocytes have also been implicated in various pathological processes; for example, in multiple sclerosis, perivascular fibrosis composed of fibrillar collagens with biglycan and decorin accumulates between the endothelium and the astrocytic glia limitans and is closely associated with infiltrating immune cells [2]. Impairment of normal astrocyte functions during stroke and other insults can critically influence neuron survival. Long-term recovery after brain injury, through neurite outgrowth, synaptic plasticity, or neuron regeneration, is also influenced by astrocyte surface molecule expression and trophic factor release [3]. Numerous studies have demonstrated that astrocytes are among the most functionally diverse group of cells in the CNS [4]. Together, studies showed astrocytes vary dramatically across regions and states, engaging distinct transcriptional programs and synapse-modulating mechanisms rather than a single "support" role. Building *in-vitro* models with functional human astrocytes offers exceptional value for mechanistic discovery and drug screening. Human Induced Pluripotent Stem Cell-derived Astrocytes (HiPSC-A) from ScienCell Research Laboratories have high purity and quality control profile to provide a robust, reliable, and consistent off-the-shelf option for human astrocytes.

HiPSC-A are cryopreserved and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. HiPSC-A are characterized by immunofluorescence with antibody specific to GFAP, S100β and Vimentin. HiPSC-A are negative for mycoplasma, bacteria, yeast, and fungi. HiPSC-A can be further cultured under the conditions provided by ScienCell Research Laboratories.

Product Content

Cat. #	# of vials	Product	Quantity	Storage
1860	1	HiPSC-A	1mL	Liquid Nitrogen
1801-100	1	Astrocyte Medium (AM)	100mL	4°C
1852	1	Astrocyte Growth Supplement (100X)	1mL	-20°C
0002-STEM	1	Fetal Bovine Serum	2mL	-20°C

Recommended Medium

It is recommended to use Astrocyte Medium (AM, Cat. #1801) for culturing HiPSC-A *in vitro*.

Additional Materials Recommended (Not provided)

Cat. #	Product	Vendor
0413	Poly-L-lysine (10 mg/mL)	ScienCell Research Laboratories
5813	CellEase	ScienCell Research Laboratories
0303	DPBS without Ca^{2+} and Mg^{2+}	ScienCell Research Laboratories
1254	ROCK Inhibitor Y-27632 (optional)	Tocris Bioscience

Product Use
HiPSC-A 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] Rudge JS. (1993) "Astrocyte-derived neurotrophic factors." In Murphy S, *Astrocytes: Pharmacology and Function* (pp 267-94). San Diego: Academic Press, Inc.
[2] van der Laan LJ, De Groot CJ, Elices MJ, Dijkstra CD. (1997) "Extracellular matrix proteins expressed by human adult astrocytes in vivo and in vitro: an astrocyte surface protein containing the CS1 domain contributes to binding of lymphoblasts." *J Neurosci Res*. 50: 539-48.
[3] Chen Y, Swanson RA. (2003) "Astrocytes and brain injury." *J Cereb Blood Flow Metab*. 23: 137-49.
[4] Shao Y, McCarhy KD. (1994) "Plasticity of astrocytes." *Glia*. 11: 147-55.

Instructions for culturing HiPSC-A cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37ºC 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.

Initiating the culture:

Note: ScienCell human induced pluripotent stem cell-derived cells must be cultured in a 37ºC, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare a poly-L-lysine-coated culture vessel (2 µg/cm^2, T-25 flask is recommended). To obtain a 2 µg/cm^2 poly-L-lysine-coated culture vessel, add 5 ml of sterile water to a T-25 flask and then add 5 µl of poly-L-lysine stock solution (10 mg/ml, Cat. #0413). Leave the vessel in a 37ºC incubator overnight (or for a minimum of one hour).
2. Prepare complete Astrocyte Medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.

   Note*: Applying ROCK inhibitor Y-27632 in the first 24 hours can improve the cell viability.*
3. Rinse the poly-L-lysine-coated vessel twice with sterile water and then add 5 ml of sterile water. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Take one vial of HiPSC-A out of the liquid nitrogen. Immediately transfer the vial into a 37ºC water bath and gently swirl it or until most of contents are thawed and only a small piece of ice remains.

   Note: *The viability of the cells will decrease if the vial contents are completely thawed.*
5. Immediately remove the vial from the water bath, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads. Using a 5 mL pipette, gently resuspend the contents of the vial homogenously. Transfer cell suspension to 15 mL tube containing 10 mL of Astrocyte Medium. Wash the emptied vial with 1 mL medium and combine with the cell suspension in the tube.

   Note: *Centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture. It is also important to minimize the time for step 3-4.*
6. Bring the poly-L-lysine-coated culture vessel to the hood and aspirate the water. Gently mix cells to get a homogenous suspension with 5 mL pipette and seed the cells into T-25 flask or equivalent.
7. Return the culture vessel to the incubator at 37ºC 5% CO_2.
8. For best results, do not disturb the culture for 16 hours after the culture has been initiated. Change the medium the next day to remove unattached cells and residual DMSO.

Maintaining the culture:

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.
2. Change the medium every three days, until the culture is approximately 70% confluent.
3. Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent.

Subculturing:

1. Subculture when the culture reaches 90-95% confluency.
2. Prepare poly-L-lysine-coated culture vessels at least 1 hour before.
3. Warm Astrocyte 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 37ºC water bath prior to use.
4. Rinse the cells with DPBS.
5. Dissociate the astrocytes with CellEase protocol.

   Note: *We recommend using CellEase which is optimized to minimize cell damage due to over digestion.*
6. Gently resuspend cells in Astrocyte Medium.
7. Count and plate cells in a new culture vessel at desired cell density for assay. A seeding density of 10,000-20,000 cells/cm^2 is recommended for expansion.

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

Caution: Handling human derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1] Grizzle WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods*. 11: 191-9.