Mouse Astrocytes-cerebellar (MA-c)

Catalog #M1810-57

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, nutritional and metabolic support throughout life [1]. Astrocytes have also been implicated in various pathological processes [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]. Much of what we have learned about astrocytes is from *in vitro* studies and astrocyte culture is a useful tool for exploring the diverse properties of this cell type.

MA-c from ScienCell Research Laboratories are isolated from postnatal day 8 C57BL/6 mouse cerebellum. MA-c are cryopreserved as primary cultures and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. MA-c are characterized by immunofluorescence with antibody specific to GFAP. MA-c are negative for mycoplasma, bacteria, yeast, and fungi. MA-c are guaranteed to further expand for 5 population doublings under the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Astrocyte Medium-animal (AM-a, Cat. #1831) for culturing MA-c *in vitro*.

Product Use

MA-c 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 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!

Initiating the culture:

1. Prepare a poly-L-lysine-coated culture vessel (2 µg/cm^2, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15 µl of poly-L-lysine stock solution (10 mg/ml, Cat. #0413). Leave the vessel in a 37°C incubator overnight (or for a minimum of one hour).
2. Prepare complete 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.
3. Rinse the poly-L-lysine-coated vessel twice with sterile water and then add 15 ml of complete medium. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. 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.
5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, poly-L-lysine-coated culture vessel. A seeding density of 5,000 cells/cm^2 is recommended.

   Note: Dilution and 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 that cells are plated in poly-L-lysine-coated culture vessels to promote cell attachment.
6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.
7. Return the culture vessel to the incubator.
8. 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.

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 thereafter, 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% confluency or above.
2. Prepare poly-$_L$-lysine-coated culture vessels (2 μg/cm^2) one day before subculture.
3. Warm complete medium, trypsin/EDTA solution (T/E, Cat. #0103), T/E neutralization solution (TNS, Cat. #0113), 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. Add 8 ml of DPBS and then 2 ml of T/E solution into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Incubate the flask in a 37ºC incubator for 1 to 2 minutes or until cells completely round up. Use a microscope to monitor the change in cell morphology.
6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).
7. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37ºC for another 1 to 2 minutes (no solution in the flask at this moment).
8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.
9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.
10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.

    Note: Use ScienCell T/E solution that is optimized to minimize cell damages due to over trypsinization.
11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Resuspend cells in culture medium.
12. Count and plate cells in a new poly-$_L$-lysine-coated culture vessel with the recommended cell density.

Caution: Handling animal-derived products is potentially biohazardous. 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.