# Mouse Astrocytes-spinal cord (MA-sc)

Catalog Number: M1830

## Cell Specification

Astrocytes are the major cell type in the mammalian brain. They have been implicated in a variety of supportive functions for their partner neurons in the CNS, such as neuronal guidance during development, ion and water homeostasis, blood flow regulation, neurotransmission, energy metabolism, and immune defense [1]. Recent studies have shown that spinal cord astrocytes contribute to neuroinflammation by chemokine expression which leads to the recruitment of "inflammatory" monocytes and neutrophils to the lesion site [2]. Experimentally, spinal cord astrocytes have also been used to study the wobbler mutation and muscular dystrophy in mice [3] and astrogliosis [4]. As the recognition of the importance of astrocytes in nervous system functioning is increasing, specifically regarding the modulation of neural activity, astrocyte cultures are continuing to provide a useful tool in exploring the diverse properties and functions of these cells.

MA-sc from ScienCell Research Laboratories are isolated from neonate day two mouse spinal cord. MA-sc are cryopreserved at primary culture and delivered frozen. Each vial contains >1 x $10^6$ cells in 1 ml volume. MA-sc are characterized by immunofluorescence with antibody specific to GFAP. MA-sc are negative for mycoplasma, bacteria, yeast and fungi. MA-sc are guaranteed to further expand for 5 population doublings in the condition provided by ScienCell Research Laboratories.

## Recommended Medium

It is recommended to use Astrocyte Medium-animal (AM-a, Cat. No. 1831) for the culturing of MA-sc *in vitro*.

## Product Use

MA-sc are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

## Storage

Transfer cells directly and immediately from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture is needed for experiments.

## Shipping

Dry ice.

**Reference**

[1] Oberheim N, Goldman S, Nedergaard M. (2012) Heterogeneity of astrocytic form and function. *Methods in Mol Biol.* 814: 23-45.

[2] Pineau I, Sun L, Bastien D, Lacroix S. (2010) Astrocytes initiate inflammation in the injured mouse spinal cord by promoting the entry of neutrophils and inflammatory monocytes in an IL-1 receptor/MyD88-depdent fashion. *Brain Behav Immun.* 24: 540-53.

[3] Hantaz-Ambroise D, Blondet B, Murawsky M, Rieger F. (1994) Abnormal astrocyte differentiation and defective cellular interactions in wobbler mouse spinal cord. *J Neurocytol*. 23: 179-92.

[4] Bhalala O, Pan L, Sahni V, McGuire T, Gruner K, Tourtellotte W, Kessler J. (2012) microRNA-21 regulates astrocytic response following spinal cord injury. *J Neurosci*. 32: 17935-47.

# Instruction for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37$^{o}$C water bath and return them to culture as quickly as possible with minimal handling!

**Set up culture after receiving the order:**

1. Prepare a poly-$_{L}$-lysine coated flask (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. No. 0413). Leave the flask in incubator overnight (minimum one hour at 37$^{o}$C incubator).

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 tube with medium to recover the entire volume.

3. Rinse the poly-$_{L}$-lysine coated flask with sterile water twice and add 10 ml of complete medium to the flask. Leave the flask in the sterile field and proceed to thaw the cryopreserved cells.

4. Place the vial in a 37$^{o}$C water bath, hold and rotate the vial gently until the contents completely thaw. Remove the vial from the water bath promptly, wipe it down with 70% ethanol and transfer it to the sterile field. Remove the cap carefully without touching the interior threads with fingers. Gently resuspend the contents of the vial using 1 ml eppendorf pipette.

5. Dispense the contents of the vial into the equilibrated, poly-$_{L}$-lysine coated culture flask. 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 cover, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.
7. Return the culture vessel to the incubator.
8. For the best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the culture medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter.

**Maintenance of 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% confluence, change medium every other day until the culture is approximately 90% confluent.

**Subculture:**

1. Subculture the cells when they are over 90% confluent.
2. Prepare poly-$_L$-lysine coated cell culture flasks (2 μg/cm$^2$).
3. Warm medium, trypsin/EDTA solution (T/E, Cat. No. 0103), trypsin neutralization solution (TNS, Cat. No. 0113), and DPBS ($Ca^{++}$ and $Mg^{++}$ free, Cat. No. 0303) to **room temperature**. We do not recommend warming the reagents and medium at 37$^o$C water bath prior use.
4. Rinse the cells with DPBS.
5. Add 8 ml of DPBS first and then 2 ml of trypsin/EDTA solution into flask (in the case of T-75 flask). Gently rock the flask to make sure that all cells are covered by trypsin/EDTA solution. Incubate the flask at 37$^o$C incubator for 1 to 2 minutes or until cells completely round up (monitored with microscope).
6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine erum (FBS, Cat. No. 0500).
7. Transfer trypsin/EDTA solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detached) and continue to incubate the flask at 37$^o$C for 1 or 2 minutes more (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 microscope to make sure that all cells detach.

9. Add 5 ml of trypsin neutralization solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of trypsin neutralization solution to collect the residual cells.
10. Examine under 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 Research Laboratories trypsin/EDTA solution that is optimized to minimize over trypsinization-induced cell damages.*
11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 min. Resuspend cells in culture medium.
12. Count and plate cells in a new, poly-$_{L}$-lysine coated flask with cell density as recommended.

    *Caution: Handling animal derived products is potentially biohazardous. Always wear gloves and safety glasses when working 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 and Polt S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. J Tissue Culture Methods. 11:191-9.