



# Astrocyte Culture kit (C57BL/6N Mouse)

(Mouse Cryopreserved Cell and culture medium)

## Cell specification

Astrocytes are one of the glial cells in the brain and spinal cord. They are the most abundant cells in the brain. They have functions, including construction of central nervous system, regulation of homeostasis of the neurons and formation of the blood-brain barrier.

Astrocyte Culture kit is formulated cryopreserved cells and culture medium optimized for astrocyte culture. It can be used for the anti-oxidization, anti-inflammation, analysis of cytokine and gliosis.

## Components

Product Name	Quantity	Amount	Storage Conditions	Stability
Astrocytes, C57BL/6N Mouse (Embryos (E16-18))	1 x 10 <sup>6</sup> cells/vial	1	Liquid Nitrogen vapor phase	6months
Astrocyte Culture Medium (Code: ASTM)	250 ml	1	-20°C Freezer	6months
			4°C	3months

**\*Shipping: dry ice**

**Culture Medium components: D-MEM/F12, FBS, antibiotic, etc.**

## Components of Medium:

ASTM is a complete medium designed for optimal culture for rat astrocytes in vitro. It is sterile, liquid basal medium (D-MEM/F12) which contain essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, inorganic salts, growth factors, hormones, fetal bovine serum, and antibiotics.

## Materials required but not provided

- Variable volume pipettes
- Culture vessels

## Precautions

- **Read the instructions carefully before starting the culture.**
- **Remove the cryovial from the dry ice package and immediately place into liquid nitrogen storage until use.**

## Protocols

- 1) Prepare culture vessels
- 2) Warm culture medium to 37°C.
- 3) Prepare a conical tube (for 50mL) added 10mL of Astrocyte culture medium.
- 4) Thaw the cryoserved Astrocytes vial in a 37°C water bath between 2minutes.

**Caution:Do not vortex the cells**

- 5) Transfer thawed cells into a 50 ml centrifuge tube containing 10 ml of Astrocyte Culture Medium.
- 6) Rinse the vial with 1ml of cell suspension.
- 7) Transfer cell suspension to appropriate flasks, petri dishes or well plates. The recommended cells density is  $0.5\sim 1 \times 10^4$ cells/cm<sup>2</sup>.

**Note: Centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual cell freezing medium in the culture and cell attachment.**

- 8) Incubate the plate at 37°C under 5% CO<sub>2</sub>, 100% humidity.
- 9) Refresh culture medium the next day to remove residual cell freezing medium and unattached cells, then every 2~3 days thereafter.

\*After 4~6 days, astrocytes reach approximately 80~100% confluent.

\*Astrocytes are weak to adhesion of glass. When culturing to a slide glass or a glass dish, the recommended cells density is  $2\sim 5 \times 10^4$ cells/cm<sup>2</sup>. Astrocytes reach approximately 80~100% confluent about one week.

## Quality Control

The Astrocytes are characterized for each lot by Immunofluorescence of Glial Fibrillary Acidic Protein(GFAP)

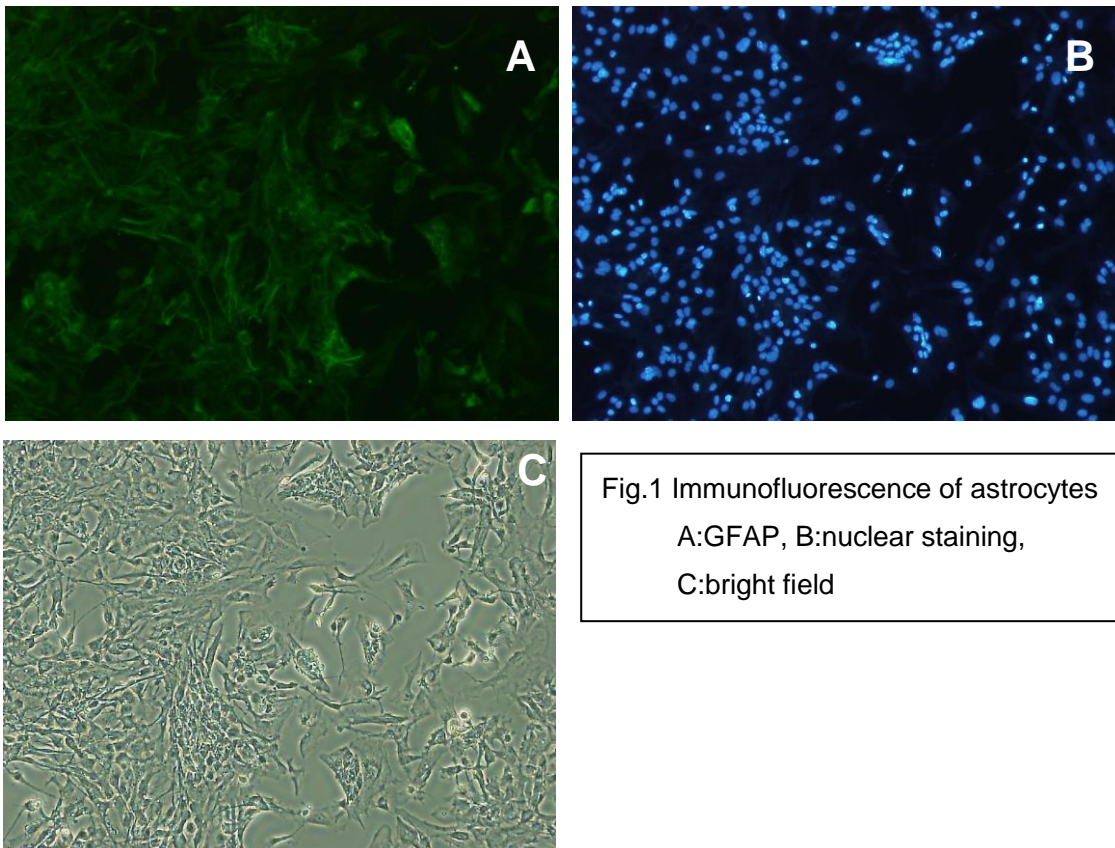


Fig.1 Immunofluorescence of astrocytes  
A:GFAP, B:nuclear staining,  
C:bright field

## Frequently Asked Questions

**Q.** How many passages can I use primary astrocytes cells?

**A.** It is not recommended that astrocytes are subcultured, as this can promote growth of contaminating cells.

**Q.** Have you measured the purity of the astrocytes?

**A.** We do not measure the purity of astrocytes. But we have confirmed by the immunostaining method that there are more than 90% of GFAP positive cells.

## References

- 1) Miller, R. H., French-Constant, C., and Raff, M. C. (1989). The macroglial cells of the rat optic nerve. *Annu. Rev. Neurosci.* 12, 517-534. (2)

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