Miltenyi Biotec

AstroMACS Medium

Order no. 130-117-031

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1. Description

This product is for research use only.

Components	**500 mL MACS® Neuro Medium** **10 mL MACS NeuroBrew®-21 (50×)** **1 vial AstroMACS Supplement, lyophilized**
Capacity	For the preparation of 500 mL complete AstroMACS Medium.
Storage	Store MACS Neuro Medium protected from light at 2–8 °C. Do not freeze. Store MACS NeuroBrew-21 (50×) and AstroMACS Supplement protected from light at –20 °C. To avoid repeated freeze-thaw cycles, store MACS NeuroBrew-21 in aliquots (recommended 1 mL) protected from light at –20 °C. The expiration date is indicated on the vial label.

1.1 Background information

The AstroMACS Medium is an optimized serum-free cell culture medium developed for the cultivation and proliferation of isolated primary neonatal and adult mouse astrocytes. It allows cell survival and proliferation in a wide range of plating densities and is optimized also for low-density plating. The medium ensures a high survival rate and viability of plated astrocytes.

1.2 Applications

- Optimal growth and cultivation of isolated primary neonatal and adult mouse astrocytes in a wide range of plating densities.

1.3 Reagent and instrument requirements

- 200 mM L-glutamine
- Cell culture dish (24-wells), e.g., Imaging Plate CG 1.5 (24 well) (# 130-098-263)
- Poly-L-lysine
- Double-distilled water (ddH_2O)

Additional requirements when working with neonatal astrocytes (≤P7)

- (Optional) Neural Tissue Dissociation Kit (P) (# 130-092-628) for preparation of single-cell suspensions from neonatal neural tissue with subsequent cell separation using the Anti-ACSA-2 MicroBead Kit, mouse (# 130-097-678) for isolation of neonatal astrocytes.
- (Optional) Neural Tissue Dissociation Kit (T) (# 130-093-231) for preparation of single-cell suspensions from neonatal neural tissue with subsequent cell separation using the Anti-GLAST (ACSA-1) MicroBead Kit, human, mouse, rat (# 130-095-825) for isolation of neonatal astrocytes.

Additional requirements when working with adult astrocytes (>P7)

- Laminin
- (Optional) Adult Brain Dissociation Kit (# 130-107-677), mouse and rat for preparation of single-cell suspensions with subsequent cell separation using the Anti-ACSA-2 MicroBeads Kit, mouse (# 130-097-678) for isolation of adult astrocytes.
- (Optional) AstroMACS Separation Buffer (# 130-117-336) for optimal cultivation results with minimum contamination of dead cells.

2. Protocol

2.1 Reconstitution

Reconstitute the lyophilized AstroMACS Supplement with 1 mL MACS Neuro Medium. For longer storage, prepare aliquots (recommended 100 µL) and store at –20 °C. Avoid repeated freeze-thaw cycles. Thaw aliquots of reconstituted AstroMACS Supplement at 2–8 °C prior to use.

2.2 Preparation of complete medium

1. Thaw MACS NeuroBrew-21 at 2–8 °C prior to use.
2. Add 0.2% of the reconstituted AstroMACS Supplement, 2% of the MACS NeuroBrew-21, and 0.25% L-glutamine (0.5 mM) to the MACS Neuro Medium, e.g., add 100 µL AstroMACS Supplement, 1 mL MACS NeuroBrew-21, and 125 µL L-glutamine to 50 mL MACS Neuro Medium.
3. Use the complete AstroMACS Medium within 2 weeks when stored at 2–8 °C. Do not freeze.

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2.3 Cultivation of neonatal astrocytes (≤P7)

▲ Pre-warm complete AstroMACS Medium to room temperature.

1. Coat the cell culture dish (24-wells) with 0.01% poly-L-lysine overnight at 37 °C.
2. Wash the dish three times with double-distilled water.
3. Dry the dish under sterile conditions.
4. Count cells and adjust to 10^4–5×10^4 cells per 50 µL pre-warmed AstroMACS Medium. Place as a drop in the middle of each well.

   ▲ **Note:** Plating density depends on the desired cultivation period and final cell density.
5. Incubate for 30 minutes at 37 °C.
6. Carefully add 450 µL of the AstroMACS Medium to each well.
7. Maintain the culture by replacing 50% of AstroMACS Medium every other day.

   ▲ **Note:** Cells are only loosely attach after the first days of cultivation.

2.4 Cultivation of adult astrocytes (>P7)

▲ For isolation of adult mouse astrocytes (>P7) refer to the special protocol "Isolation and cultivation of astrocytes from adult mouse brain" available at www.miltenyibiotec.com/astroprotocol.

▲ Pre-warm complete AstroMACS Medium to room temperature.

1. Coat the cell culture dish (24-well plate) with 0.01% poly-L-lysine overnight at 37 °C.
2. Wash the dish three times with double-distilled water.
3. Coat the cell culture dish (24-well plate) with 10 µg/mL laminin overnight at 37 °C.
4. Wash the dish three times with double-distilled water.
5. Dry the dish under sterile conditions.
6. Count cells and adjust to 10^5 cells per 50 µL pre-warmed AstroMACS Medium. Place as a drop in the middle of each well.
7. Incubate for 30 minutes at 37 °C.
8. Carefully add 450 µL of the AstroMACS Medium to each well.
9. Maintain the culture by replacing 50% of AstroMACS Medium every other day.

   ▲ **Note:** Cells only loosely attach after the first days of cultivation.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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