# Primary Mouse Astrocyte Isolation

Trypsin-based isolation of astrocytes from neonatal or adult mouse brain

## Overview

This protocol describes the isolation and culture of primary astrocytes from mouse brain. Astrocytes are the most abundant glial cell type in the brain and play critical roles in neuronal support, synaptic function, and CNS homeostasis.

### Workflow Summary:

- **Step 1:** Euthanize mouse and harvest brain
- **Step 2:** Remove meninges
- **Step 3:** Trypsinization (15 min)
- **Step 4:** Trituration and neutralization
- **Step 5:** Centrifugation and plating
- **Duration:** ~1-2 hours from dissection to plating
- **Culture time:** 7-14 days for confluent astrocytes

### Applications

- Neuron-astrocyte co-culture studies
- Inflammatory response assays
- Metabolic studies
- Reactive astrogliosis modeling
- Drug screening
- Astrocyte-specific gene expression

### Key Features

- Simple trypsin-based dissociation
- High purity (>95% GFAP+)
- Best from P0-P2 neonatal mice
- Can be used from adult mice (lower yield)
- Cells maintain astrocyte phenotype

• Can be passaged 2-3 times

### Age Considerations:

- **P0-P2 (neonatal):** Highest yield, easiest dissociation, preferred
- **P7-P10:** Good yield, slightly more difficult
- **Adult (>P21):** Lower yield, requires longer digestion, more debris

### Safety & Animal Care

- Ensure proper IACUC approval for all animal procedures
- Follow institutional guidelines for animal euthanasia
- Work in biosafety cabinet for sterile technique
- Wear appropriate PPE: lab coat, gloves, eye protection
- Dispose of animal carcasses according to institutional policy
- Genotyping: Save tail clip if needed for genotyping

## Required Materials

### Reagents

- DMEM high glucose (DMEM HG)
- Fetal bovine serum (FBS) or primary cell serum
- Penicillin/Streptomycin
- L-Glutamine or GlutaMAX
- 0.25% Trypsin-EDTA or 0.05% Trypsin
- PBS (phosphate buffered saline)
- 70% ethanol (for sterilization)

### Equipment & Supplies

- Biological safety cabinet (BSC)
- 37°C $CO_2$ incubator
- 37°C water bath
- Centrifuge
- Dissection microscope (optional but helpful)

- Dissection tools (scissors, forceps)
- 10 cm Petri dishes (sterile)
- 6 cm culture dishes
- 15 mL conical tubes
- Pipettes (serological and micropipettes)
- Paper towels
- Dry ice
- Eppendorf tubes (for tail clips)

### Media Recipes

**Dissection Medium:**

- DMEM high glucose
- No serum (plain DMEM)
- Keep on ice during dissection

**Astrocyte Culture Medium:**

- DMEM high glucose
- 10% FBS (or primary cell serum)
- 1% Penicillin/Streptomycin
- 1% L-Glutamine or GlutaMAX
- Sterile filter (0.22 µm)

### Animals

- **Preferred:** P0-P2 neonatal mice
- **Alternative:** P7-P10 or adult mice
- Can use any mouse strain
- Typical: 1-3 pups per preparation
- Expected yield: ~1-2 × $10^6$ cells per brain

## Astrocyte Isolation Protocol

> **Timing: ~1-2 hours from euthanasia to plating**

### Step 1: Preparation

Mark Done

- Pre-warm culture medium to 37°C

- Pre-warm water bath to 37°C
- Prepare ice bucket with dissection medium (DMEM HG, no serum)
- Sterilize dissection tools (autoclave or 70% ethanol)
- Set up biosafety cabinet with supplies
- Label 15 mL tubes (one per animal)
- Add 2 mL trypsin to each labeled tube, keep on ice

## Step 2: Euthanasia and Brain Harvest

Mark Done

- Place mouse on paper towel on dry ice for rapid euthanasia
- Wait until mouse stops moving (hypothermia-induced euthanasia)
- **Alternative:** Use $CO_2$ or cervical dislocation per IACUC protocol
- Decapitate mouse with sharp scissors
- **If needed for genotyping:** Cut tail clip and place in labeled Eppendorf tube
- Spray head with 70% ethanol

## Step 3: Brain Extraction

Mark Done

- Using scissors, cut skin from back of head toward nose
- Peel skin away to expose skull
- Insert scissors into foramen magnum (base of skull)
- Cut along midline from back to front of skull
- Carefully peel skull away using forceps
- Gently scoop brain out with curved forceps or spatula
- Place brain in 10 cm dish with ice-cold DMEM HG

## Step 4: Meninges Removal

Mark Done

- Transfer brain to fresh DMEM in 10 cm dish
- Using fine forceps, carefully peel away meninges (thin membrane covering brain)
- Meninges appear as translucent tissue with blood vessels
- Work under dissection microscope if available
- **Critical:** Complete meninges removal prevents fibroblast contamination
- For neonatal brains: meninges are very soft and thin
- Rinse brain gently in DMEM to remove remaining debris

## Step 5: Trypsinization

Mark Done

- Transfer cleaned brain to labeled 15 mL tube containing 2 mL trypsin
- Keep tube on ice until ready for digestion
- Place tube in 37°C water bath for **15 minutes**
- Flick tube every 5 minutes to mix and ensure even digestion
- Brain tissue should start to look "softer" and more dissociated

### Step 6: Trituration

Mark Done

- Remove tube from water bath
- Triturate (pipette up and down) for **1 minute** using 5 mL pipette
- Pipette gently at first, then more vigorously
- Break up any large clumps of tissue
- Solution should become cloudy with single-cell suspension

### Step 7: Neutralization and Washing

Mark Done

- Add 8 mL DMEM HG containing serum (10% FBS) to neutralize trypsin
- Mix gently by inverting tube several times
- Final volume: ~10 mL

### Step 8: Centrifugation

Mark Done

- Centrifuge tube at **2,000 RPM (400 × g) for 10 minutes** at room temperature
- Cell pellet should be visible at bottom of tube
- Carefully decant supernatant without disturbing pellet

### Step 9: Resuspension and Plating

Mark Done

- Add 3 mL DMEM HG + serum medium to pellet
- Resuspend pellet by pipetting gently up and down
- Transfer entire cell suspension to labeled 6 cm culture dish
- Swirl gently to distribute cells evenly
- Place dish in 37°C, 5% $CO_2$ incubator

### Step 10: Initial Culture

Mark Done

- Do not disturb cells for first 24 hours
- Cells will begin attaching within 2-4 hours
- After 24 hours, check cells under microscope
- You should see mixed population: astrocytes (flat, process-bearing), neurons (small, round), microglia (small, ramified)
- Change medium after 48 hours to remove debris and non-adherent cells

## Astrocyte Culture and Maintenance

> **Astrocyte Enrichment Strategy**
>
> Initial cultures contain mixed cell types. Astrocytes proliferate and overgrow other cell types within 7-14 days, resulting in >95% purity.

### Day 1-3: Initial Culture

- Mixed population of cells will attach
- Change medium on Day 2 or 3 to remove debris
- Gently aspirate medium and add 3 mL fresh astrocyte medium

### Day 4-7: Astrocyte Expansion

- Astrocytes begin to proliferate
- Change medium every 2-3 days
- Cells will form flat, polygonal morphology
- Some neurons and microglia still present

### Day 7-14: Confluence and Purification

- Astrocytes become confluent (cover entire dish surface)
- Other cell types are overgrown or detach
- Culture is now >95% astrocytes (GFAP+)
- Cells ready for experiments or passaging

### Passaging Astrocytes (Optional)

- Astrocytes can be passaged 2-3 times while maintaining phenotype
- Passage when cells reach 100% confluence
- Use 0.05% or 0.25% trypsin for 3-5 minutes at 37°C
- Split 1:3 to 1:5 ratio
- Higher passages may lose astrocyte markers and become more reactive

### Freezing Astrocytes

- Harvest confluent astrocytes by trypsinization
- Resuspend in freezing medium: 90% FBS + 10% DMSO
- Freeze at 1-2 × $10^6$ cells/mL in cryovials
- Use controlled-rate freezing (-1°C/min) to -80°C
- Transfer to liquid nitrogen for long-term storage

### Astrocyte Characterization

- **Morphology:** Flat, polygonal, stellate with processes
- **GFAP:** Glial fibrillary acidic protein (astrocyte marker)
- **S100β:** Calcium-binding protein (astrocyte marker)
- **Glutamine synthetase:** Enzyme marker
- **Negative for:** Iba1 (microglia), NeuN (neurons), O4 (oligodendrocytes)

# Troubleshooting

| Problem | Possible Cause | Solution |
|---|---|---|
| Low cell yield | Old mice<br>Incomplete dissociation<br>Meninges not removed | Use P0-P2 neonatal pups<br>Increase trituration time<br>Pool multiple brains<br>Ensure complete meninges removal |
| High fibroblast contamination | Meninges not fully removed<br>Adult mice used | Improve meninges removal technique<br>Use younger animals<br>Consider differential adhesion method |
| Cells not attaching | Over-trypsinization<br>Cell death<br>Uncoated dishes | Reduce trypsin time to 10-12 min<br>Work quickly, keep cells cold<br>Pre-coat dishes with poly-L-lysine |
| Slow astrocyte growth | Low serum quality<br>Wrong medium<br>Contamination | Use high-quality FBS (test different lots)<br>Use DMEM high glucose<br>Check for mycoplasma |
| Cells detaching after confluence | Overconfluence<br>Medium not changed regularly | Passage or use cells at 90-100% confluence<br>Change medium every 2-3 days<br>Don't let cells become overgrown |
| High microglia contamination | Normal in mixed culture<br>Adult brain source | Microglia will be diluted out with passaging<br>Shake flasks (200 RPM, 2h) to dislodge microglia<br>Use FACS to sort GFAP+ cells |

## Tips for Success

- Use P0-P2 neonatal pups for highest yield and purity
- Work quickly during dissection (keep tissue cold)
- Complete meninges removal is critical

- Don't over-trypsinize (15 min is usually sufficient)
- Be patient - astrocytes take 7-14 days to reach purity
- Use high-quality, tested FBS
- Change medium regularly to remove debris

## Quality Control

- Check cell morphology daily under microscope
- Verify GFAP expression by immunocytochemistry
- Should have >95% GFAP+ cells after 10-14 days
- Test for mycoplasma contamination
- Monitor growth rate (should double every 2-3 days)
- Use cells at passage 1-3 for best phenotype