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Rat primary cortical astrocytes

USER GUIDE

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ThermoFisher SCIENTIFIC

Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0001664

Revision	Date	Description
2.0	17 February 2020	Updated to thawing instructions
1.0	1 May 2009	New document

Contents

Contents and storage

Shipping and storage

Rat Primary Cortical Astrocytes are shipped on dry ice. Upon receipt, store the cells in **liquid nitrogen, vapor-phase**.

Contents

Amount supplied: One vial containing 1×10^6 viable cells.

Composition: 1 mL of cells in freezing medium.

Note: Freezing medium: 90% Astrocyte growth medium (85% Dulbecco's Modified Eagle medium containing 4.5 g/L glucose, and 15% Fetal Bovine Serum) plus 10% DMSO.

CAUTION! Handle cells as potentially biohazardous material under at least Biosafety Level 1 (BL-1) containment. This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Material Safety Data Sheet (MSDS) before handling. Material Safety Data Sheets (MSDSs) are available on our website.

Important guidelines for thawing and storing cells

- Upon receipt, immediately thaw cells or place into vapor-phase liquid nitrogen storage until ready to use. **Do not store the cells at –80°C**.
- Avoid short-term extreme temperature changes. When storing cells in liquid nitrogen after shipping on dry ice, allow the cells to remain in liquid nitrogen for 3-4 days before thawing.

Additional products

The products listed in this section may be used with Rat Primary Cortical Astrocytes. For more information, refer to our website.

Item	Quantity	Cat. no.
Dulbecco's Modified Eagle Medium (D-MEM™) (1X), liquid (high glucose)	500 mL 1000 mL	11995-065 11995-040
Fetal Bovine Serum (FBS), Certified	100 mL	16000-036
Dulbecco's Phosphate Buffered Saline (D-PBS), containing no calcium, magnesium, or phenol red	500 mL	14190-144
Dulbecco's Phosphate Buffered Saline (D-PBS), containing calcium and magnesium, but no phenol red	500 mL	14040-133
StemPro™ Accutase™ Cell Dissociation Reagent	100 mL	A11105-01
Trypan Blue Stain	100 mL	15250-061
Trypan Blue Stain 0.4% (for use with the Countess™ Automated Cell Counter)	2 × 1 mL	T10282
LIVE/DEAD™ Cell Vitality Assay Kit	1000 assays	L34951
Countess™ Automated Cell Counter (includes 50 Countess™ cell counting chamber slides and 2 mL of Trypan Blue Stain)	1 unit	C10227

Products for marker analysis

The products listed below may be used for analyzing the phenotype of Rat Primary Cortical Astrocytes. In addition to the primary antibodies listed below, we offer a variety of isotype specific secondary antibodies conjugated with enzymatic and fluorescent indicators, as well as antibody sera and diluents. For more information, refer to our website.

Item	Quantity	Cat. no.
Rabbit anti-Doublecortin (DCX)	100 µg	48-1200
Rabbit anti-GFAP (Glial Fibrillary Acid Protein)	1 mL	18-0063
DAPI (4′,6-diamidino-2-phenylindole, dihydrochloride)	10 mg	D1306
ProLong™ Gold Antifade Reagent	10 mL	P36930
ProLong™ Gold Antifade Reagent with DAPI	10 mL	P36931

Introduction

Rat primary cortical astrocytes

Introduction

Astrocytes are by far the most numerous cell type in the central nervous system (CNS), outnumbering their neuronal counterparts by approximately tenfold, and have critical roles in adult CNS homeostasis (Pekny & Nilsson, 2005). They provide biochemical and nutritional support of neurons and endothelial cells which form the blood-brain barrier, perform the vast majority of synaptic glutamate uptake, and maintain extracellular potassium levels (Rothstein *et al*., 1996; Rothstein *et al*., 1994). Astroglial dysfunction has been implicated in a number of CNS pathologies including amyotrophic lateral sclerosis (ALS) and ischemic neuronal death (Maragakis & Rothstein, 2006; Takano *et al*., 2009), and transplantation-based astrocyte replacement therapy has been shown to be a promising therapeutic strategy against neuronal death (Lepore *et al*., 2008). Although there are few known differences between cortical and hippocampal astrocytes, it has been reported that astrocytes from different regions of the brain show a differential sensitivity to ischemic injury (Xu *et al*., 2001; Zhao & Flavin, 2000).

Source of rat primary cortical astrocytes

Rat Primary Cortical Astrocytes are isolated from the cortices of fetal Sprague-Dawley™ rats at embryonic day 19 (E19) of gestation. The cells are isolated from tissue under sterile conditions, placed through one round of enzymatic dissociation and expansion in astrocyte growth medium (85% Dulbecco's Modified Eagle medium containing 4.5 g/L glucose, and 15% Fetal Bovine Serum). The cells are cryopreserved at passage 1 (P1) in 90% astrocyte growth medium plus 10% DMSO. Each vial of Rat Primary Cortical Astrocytes contains 1×10^6 cells/mL that can be expanded in culture for at least one passage.

Characteristics of rat primary cortical astrocytes

- Isolated from the brain cortex of fetal Sprague-Dawley™ rats at embryonic day 19 (E19) of gestation
- Exhibit ≥ 70% viability upon thawing
- Stain > 80% positive for the astrocyte specific marker, glial fibrillary acid protein (GFAP)
- Stain ≤ 10% positive for neuron and oligodendrocyte specific markers galactocerebroside (GalC) and doublecortin (DCX)
- Exhibit a doubling time of approximately 9 days at P2
- Can be expanded in culture for at least one passage

Rat cortical astrocyte culture

Primary cells isolated from the cortex of fetal Sprague-Dawley™ rat can be expanded for at least one passage in culture. The image below shows Rat Primary Cortical Astrocytes at day five after plating.

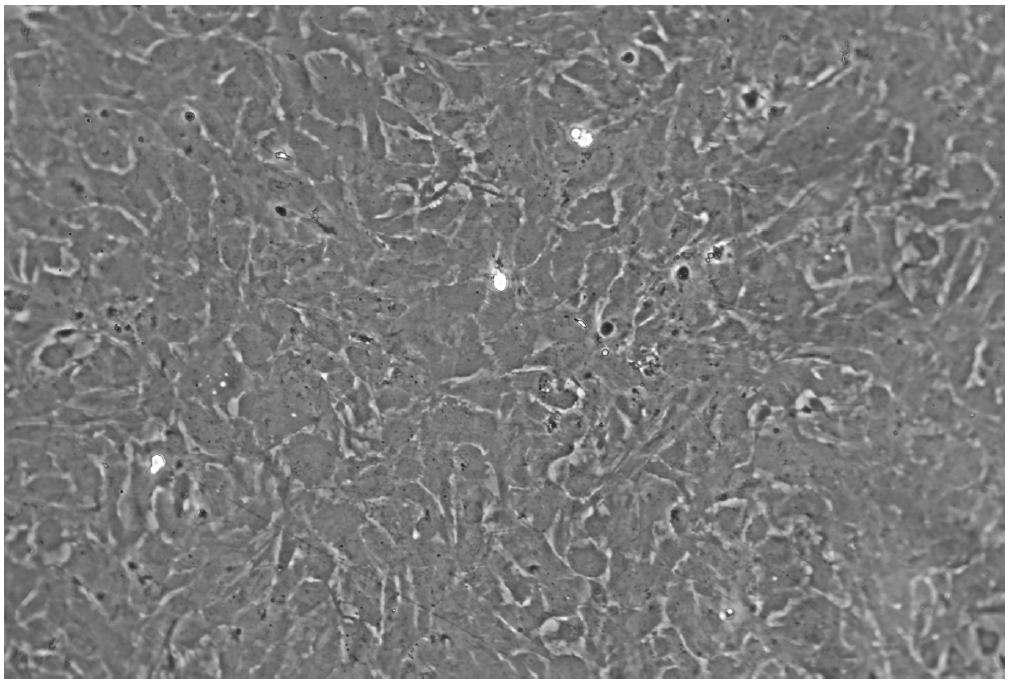

Figure 1 Bright field image of adherent Rat Primary Cortical Astrocytes at passage 2 (P2) that have been cultured in astrocyte growth medium for five days. The image was captured using 10X objective lens.

Methods

Handling rat primary cortical astrocytes

CAUTION! As with other mammalian cells, when working with Rat Primary Cortical Astrocytes, handle as potentially biohazardous material under at least Biosafety Level 1 (BL-1) containment. For more information on BL-1 guidelines, refer to *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed., published by the Centers for Disease Control, or see the following website: **www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm**

Guidelines for culturing rat primary cortical astrocytes

Follow the general guidelines below to grow and maintain Rat Primary Cortical Astrocytes.

- **All solutions and equipment that come in contact with the cells must be sterile**. Always use proper aseptic technique and work in a laminar flow hood.
- For consistent results in your experiments, we recommend using cells below passage 3 (P3). If you expand Rat Primary Cortical Astrocytes beyond P3, we recommend that you perform another round of characterization prior to further experiments.
- For general maintenance of Rat Primary Cortical Astrocytes as an adherent culture, passage cells when they reach 100% confluency at a seeding density of 20,000 cells/cm^2.
- When thawing or subculturing cells, transfer cells into pre-warmed medium.
- Standard physical growth conditions for Rat Primary Cortical Astrocytes are 37°C in a humidified atmosphere of 5% CO_2 in air.

IMPORTANT! We recommend that you use Rat Primary Cortical Astrocytes right after recovery. After thawing Rat Primary Cortical Astrocytes, expand the cells once to have a 1.5 to 2-fold increase in their number, and harvest them to use in your experiments (e.g., transplantation experiments, metabolic studies).

Media requirements

Astrocyte growth medium

You can grow Rat Primary Cortical Astrocytes as an adherent culture on uncoated, tissue culture treated vessels. For optimal growth and expansion of these cells, we recommend using astrocyte growth medium consisting of 85% Dulbecco's Modified Eagle medium (containing 4.5 g/L glucose) and 15% Fetal Bovine Serum. This medium is designed to support isolation and growth of astrocytes derived from cortical tissue of fetal rats.

- Prepare your growth medium prior to use.
- When thawing or subculturing cells, transfer cells into pre-warmed medium at 37°C.
- We recommend that you aliquot astrocyte growth medium into required working amounts to avoid exposing it to 37°C multiple times.

Component	Cat. no.	Amount
Dulbecco's Modified Eagle medium (high glucose)	11995-065	85%
Fetal Bovine Serum	16000-036	15%

Thawing rat primary cortical astrocytes

Materials needed

The following materials are required (see "Additional products" on page 6 for ordering information).

- Rat Primary Cortical Astrocytes, stored in liquid nitrogen
- Ethanol or 70% isopropanol
- Astrocyte growth medium (see "Astrocyte growth medium" on page 11); pre-warmed to 37°C
- Disposable, sterile 15-mL tubes
- Flame-polished and autoclaved glass Pasteur pipettes, or plastic Pasteur pipettes pre-rinsed with growth medium
- 37°C water bath
- 37°C incubator with a humidified atmosphere of 5% CO_2
- Microcentrifuge
- Tissue-culture treated flasks, plates, or Petri dishes (uncoated)
- Hemacytometer, cell counter and Trypan Blue, LIVE/DEAD™ Cell Vitality Assay Kit, or the Countess™ Automated Cell Counter

Note: The Countess™ Automated Cell Counter is a benchtop instrument designed to measure cell count and viability (live, dead, and total cells) accurately and precisely in less than a minute per sample, using the standard Trypan Blue uptake technique (see "Additional products" on page 6 for ordering information).

Using the same amount of sample that you currently use with the hemocytometer, the Countess™ Automated Cell Counter takes less than a minute per sample for a typical cell count and is compatible with a wide variety of eukaryotic cells.

IMPORTANT! Rat Primary Cortical Astrocytes readily stick to the plastic used in cell culture dishes and centrifuge tubes. Prior to use, rinse all material that will come in contact with the cells with medium to prevent cells from sticking to the plastic. To thaw and establish Rat Primary Cortical Astrocytes, follow the

Thawing procedure

1. Remove the cells from liquid nitrogen storage, and **immediately** transfer the cells to a 37℃ water bath to prevent crystal formation.
2. Quickly thaw the vial of cells by gently swirling it in the 37℃ water bath and removing it when the last bit of ice has melted, typically < 2 minutes. Do not submerge the vial completely. Do not thaw the cells for longer than 2 minutes. Do not introduce bubbles into the cell suspension as it decreases cell viability.
3. When thawed, transfer the tube containing the cells into the laminar flow hood, and wash the outside of the tube with 70% isopropanol.
4. Very gently transfer the cells into a pre-rinsed 15-mL centrifuge tube using a Flame-polished and autoclaved glass Pasteur pipette, or a pre-rinsed plastic Pasteur pipette.
5. Rinse the vial with 1 mL of astrocyte growth medium, and dropwise add to the cells in the 15-mL centrifuge tube (one drop/second). Mix by gentle swirling after each drop.
6. Dropwise add 8 mL of astrocyte growth medium to the cell solution and mix gently.
7. Centrifuge the cells at 250 × g for 5 minutes.
8. Aspirate the supernatant and resuspend cells in 2 mL of astrocyte growth medium.
9. Determine the viable cell count using your method of choice.
10. Plate the cells at a seeding density of 2×10^4 cells per cm^2 on an uncoated, tissue-culture treated culture dish. If necessary, gently add growth medium to the cells to achieve the desired cell concentration and recount the cells.
11. Incubate at 37℃, 5% CO_2, and 90% humidity. Replace the medium with an equal volume of fresh, pre-warmed astrocyte growth medium every 4–5 days.
12. Passage cells when the culture is 100% confluent.

Expanding rat primary cortical astrocytes

Introduction

You may expand Rat Primary Cortical Astrocytes as an adherent culture on uncoated, tissue-culture treated flasks, plates or dishes. Subculture your cells when 100% confluent.

Note: We recommend that you use Rat Primary Cortical Astrocytes right after recovery. After thawing Rat Primary Cortical Astrocytes, expand the cells once to have a 1.5 to 2-fold increase in their number, and harvest them to use in your experiments (*e.g.*, transplantation experiments, metabolic studies).

Materials needed

The following materials are required for passaging Rat Primary Cortical Astrocytes (see "Additional products" on page 6 for ordering information).

- Culture vessels containing Rat Primary Cortical Astrocytes (100% confluent)
- Uncoated, tissue-culture treated flasks, plates, or Petri dishes
- Astrocyte growth medium (see "Astrocyte growth medium" on page 11), pre-warmed to 37℃
- Disposable, sterile 15-mL or 50-mL conical tubes, pre-rinsed with medium
- 37℃ incubator with humidified atmosphere of 5% CO_2
- Dulbecco's Phosphate Buffered Saline (D-PBS), containing no calcium, magnesium, or phenol red
- StemPro™ Accutase™ Cell Dissociation Reagent (see "Additional products" on page 6), pre-warmed to 37℃
- Hemacytometer, cell counter and Trypan Blue, LIVE/DEAD™ Cell Vitality Assay Kit, or the Countess™ Automated Cell Counter

IMPORTANT! Rat Primary Cortical Astrocytes readily stick to the plastic used in cell culture dishes and centrifuge tubes. Prior to use, rinse all material that will come in contact with the cells with medium to prevent cells from sticking to the plastic. To passage Rat Primary Cortical Astrocytes, follow the procedure on the next page.

Passaging rat primary cortical astrocytes

1. Remove the spent growth medium from the culture dish containing the cells, and store in a sterile tube to use as a washing solution.
2. Rinse the surface of the cell layer once with D-PBS without Ca^{2+} and Mg^{2+} (approximately 2 mL D-PBS per 10 cm^2 culture surface area) by adding the D-PBS to the side of the vessel opposite the attached cell layer, and rocking back and forth several times.
3. Aspirate the D-PBS and discard.
4. To detach the cells, add 3 mL of pre-warmed StemPro™ Accutase™ Cell Dissociation Reagent per T75 flask; adjust volume accordingly for culture dishes of other sizes.

5. Incubate for up to 20 minutes at 37℃. Rock the cells every 5 minutes, and check for cell detachment and dissociation toward single cell under the microscope.

6. Once you observe cell detachment, gently pipette up and down to break clumps into a single cell suspension. Stop the cell dissociation reaction by an adding equal volume of the spent medium from step 1 on page 13. Disperse the medium by pipetteting over the cell layer surface several times.

7. Transfer the cells to a new 15-mL or 50-mL pre-rinsed conical tube, and centrifuge at 250 × *g* for 5 minutes at room temperature. Aspirate and discard the supernatant.

8. Gently resuspend the cell pellet in pre-warmed astrocyte growth medium and remove a sample for counting.

9. Determine the total number of cells and percent viability using your method of choice. If necessary, add astrocyte growth medium to the cells to achieve the desired cell concentration and recount the cells.

10. Plate cells in an uncoated tissue-culture treated flask, plate, or Petri dish at a seeding density of 2×10^4 cells per cm^2.

11. Incubate cells at 37℃, 5% CO_2, and 90% humidity, and change growth medium every 4–5 days.

Characterizing phenotype of rat primary cortical astrocytes

Phenotypic markers

Immunocytochemical analysis of Rat Primary Cortical Astrocytes using fluorochrome-conjugated antibodies to astrocyte-specific marker GFAP should indicate ≥ 80% expression, while the expression of the neuron-specific marker DCX and the oligodendrocyte-specific marker GalC should be ≤ 10%.

See Figure 2 on page 17 for an example of phenotypic marker expression of Rat Primary Cortical Astrocytes cultured in astrocyte growth medium and analyzed using the protocol on the next page.

Primary antibodies

The following table lists the primary antibodies used for classifying Rat Primary Cortical Astrocytes. See "Products for marker analysis" on page 7 for ordering information.

Note: The behavior of the antibodies and their dilution ratio is dependent on their source and concentration. We recommend that you optimize the parameters of your immunocytochemistry experiments (*e.g.*, dilution ratio, incubation time) if you use antibodies from a source other than listed below.

Cell Type	Antigen	Dilution ratio	Antibody type
Astrocytes	GFAP (Invitrogen™, Cat. no. 18-0063)	1:200	Rabbit IgG
Neurons	DCX (Invitrogen™, Cat. no. 48-1200)	1:400	Rabbit IgG
Oligodendrocytes	GalC (Millipore™, Cat. no. MAB342)	1:200	Mouse IgG

Immunocytochemistry

Fixing Cells:

1. Remove culture medium and gently rinse the cells once with D-PBS without dislodging.
2. Fix the cells with 4% fresh **Paraformaldehyde Fixing Solution** (PFA; see Appendix A, "Appendix", "Paraformaldehyde solution" on page 20 for recipe) at room temperature for 15 minutes.
3. Rinse 3X with D-PBS containing Ca^{2+} and Mg^{2+}.
4. Check for presence of cells after fixing.
5. Proceed to staining on the next page. You may also store slides for up to 3–4 weeks in D-PBS at 4℃. **Do not** allow slides to dry.

Staining Cells:

1. Incubate cells for 30–60 minutes in blocking buffer (5% serum of the secondary antibody host species, 1% BSA, 0.1% Triton-X™ in D-PBS with Ca^{2+} and Mg^{2+}).

   Note: If you are using a surface antigen such as GalC, omit Triton-X™ in blocking buffer.
2. Remove the blocking buffer and incubate cells overnight at 4°C with primary antibody diluted in D-PBS containing 5% serum. Ensure that the cell surfaces are covered uniformly with the antibody solution.
3. Wash the cells 3X for 5 minutes with D-PBS containing Ca^{2+} and Mg^{2+} (if using a slide, use a staining dish with a magnetic stirrer).
4. Incubate the cells with fluorescence-labeled secondary antibody (5% serum in D-PBS with Ca^{2+} and Mg^{2+}) **in the dark** at 37°C for 30–45 minutes.
5. Wash the cells 3X with D-PBS containing Ca^{2+} and Mg^{2+}, and in the last wash counter stain with DAPI solution (3 ng/mL) for 5 minutes, and rinse with D-PBS.
6. If desired, mount with 3 drops of ProLong™ Gold antifade reagent per slide and seal with the cover slip (see “Products for marker analysis” on page 7 for ordering information). You may store the slides **in the dark** at 4°C.

Phenotype marker expression of rat primary cortical astrocytes

Astrocyte-specific marker expression

The image below shows the phenotype marker expression of Rat Primary Cortical Astrocytes cultured in astrocyte growth medium for eleven days, and analyzed using the protocol on the previous page.

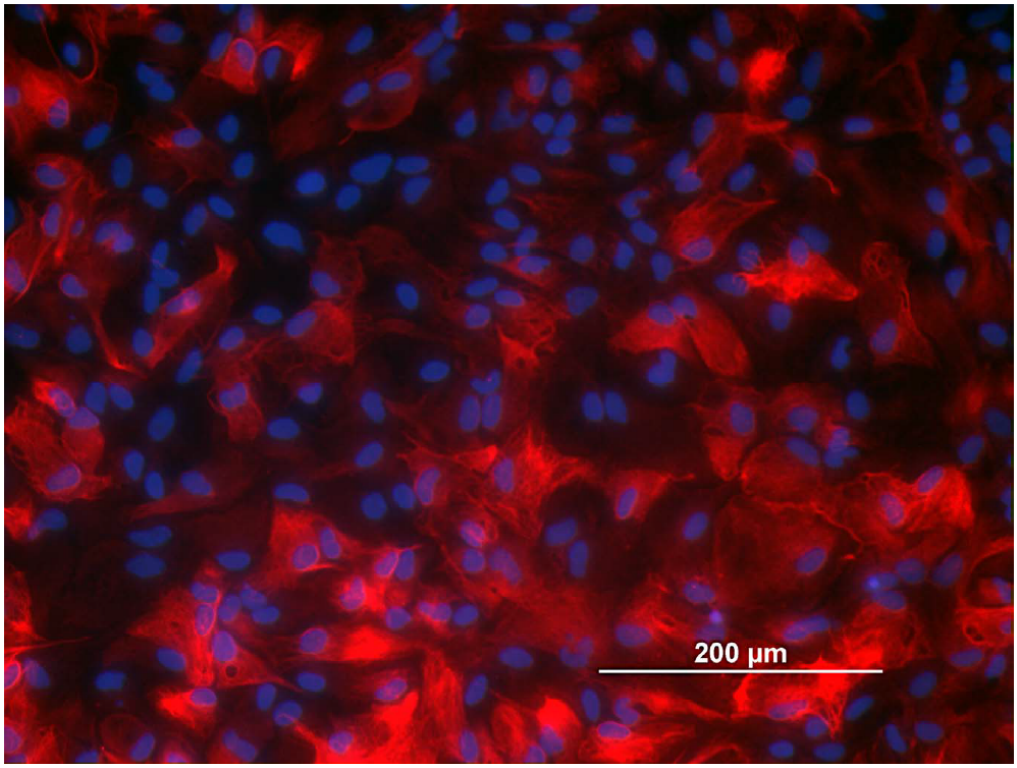

Figure 2 Rat Primary Cortical Astrocytes stained by indirect immunofluorescence for the intracellular marker GFAP (red). Nuclei were stained with DAPI (blue). The cells were maintained in astrocyte growth medium (85% Dulbecco's Modified Eagle medium containing 4.5 g/L glucose, and 15% Fetal Bovine Serum) for eleven days prior to 4% paraformaldehyde fixation and staining. While ≥ 80% of the cells stain positive for GFAP, ≤ 10% of the cells show neuron-specific DCX and oligodendrocyte-specific GalC expression (data not shown). Scale bar = 200 µm.

Troubleshooting

Culturing cells

The table below lists some potential problems and solutions that help you troubleshoot your cell culture problems.

Problem	Cause	Solution
No viable cells after thawing stock	Stock not stored correctly	Order new stock and store in liquid nitrogen. Keep in liquid nitrogen until thawing.
	Cell not handled gently	Follow procedures in **Thawing Rat Primary Cortical Astrocytes** ("Thawing rat primary cortical astrocytes" on page 11) exactly. Fast thawing is the key for a healthy culture. Add medium in drop-wise manner (slowly). At time of thawing, thaw quickly and do not expose vial to the air but quickly change from nitrogen tank to 37℃ water bath.
		Obtain new Rat Primary Cortical Astrocytes.
		Use pre-warmed complete growth medium, prepared as described in "Astrocyte growth medium" on page 11.
		Generally we recommend a culture density of 2×10^4 cells per cm^2 at the time of recovery.
	Thawing medium not correct	Rat Primary Cortical Astrocytes are fragile; treat your cells gently, do not vortex, bang the flasks to dislodge the cells, or centrifuge the cells at high speeds.

Problem	Cause	Solution
Fewer viable cells than expected after thawing stock	Cells sticking to plastic culture vessel or pipette tip	Prior to use, rinse all material that will come in contact with the cells with medium to prevent cells from sticking to the plastic.
Cells grow slowly or stop growing	Growth medium not correct	We recommend using astrocyte growth medium for optimal growth and expansion (see “Astrocyte growth medium” on page 11).
	Poor serum in growth medium	Use Fetal Bovine Serum from a different lot, and do not heat inactivate serum.
	Cells have been passaged too many times	Obtain new, P1 Rat Primary Cortical Astrocytes.

Appendix

Paraformaldehyde solution

To prepare 20% paraformaldehyde (PFA) stock solution:

1. Add PBS to 20 g of EM grade paraformaldehyde (Electron Microscopy Services, Cat. no. 19208), and bring the volume up to 100 mL.
2. Add 0.25 mL of 10 N NaOH and heat at 60°C using a magnetic stirrer until completely dissolved.
3. Filter through 0.22 micron filter, and cool on ice. Make sure the pH is 7.5–8.0.
4. Aliquot 2 mL in 15-mL tubes, freeze on dry ice, and store at –20°C.

To prepare 4% PFA for fixing:

1. Add 8 mL PBS into each 15-mL tube containing 2 mL of 20% PFA, and thaw in a 37°C water bath.
2. Once dissolved, cool on ice.

References

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Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.