

Rat glial precursor cells (GPCs)

USER GUIDE

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Contents and storage

Shipping and storage

Rat Glial Precursor Cells (GPCs) are shipped on dry ice. Upon receipt, store the cells in **liquid nitrogen, vapor-phase**.

Contents

Amount supplied: One vial containing 1×10^6 cells. Composition: 1 mL of cells in freezing medium.

Note: Freezing medium: Complete StemPro™ NSC SFM™ with 2 mM GlutaMAX™-I and 10 ng/mL Platelet Derived Growth Factor AA Homodimer (PDGF-AA), 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 Glial Precursor Cells (GPCs). For more information, refer to our website.

Item	Quantity	Cat. no.
StemPro™ NSC SFM™ (contains KnockOut™ DMEM/F-12, FGF Basic Recombinant Human, EGF Recombinant Human, and StemPro™ NSC SFM™ Supplement)	1 kit	A1050901
GlutaMAX™ -I Supplement	100 mL	35050-061
KnockOut™ DMEM/F-12	500 mL	12660-012
FGF Basic Recombinant Human (bFGF)	10 µg	PHG0024
EGF Recombinant Human	10 µg	PHG0314
PDGF-AA, Recombinant Human	10 µg	PHG0035
Fetal Bovine Serum (FBS), ES Cell-Qualified	100 mL 500 mL	16141-061 16141-079
BSA, 10% Stock Solution	25 mL	P2489
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
Dulbecco's Modified Eagle Medium (D-MEM™) (1X), liquid (high glucose)	1000 mL	11995-040
CELLStart™ Defined, Humanized Substrate for Cell Culture	2 mL	10142-01
Natural Mouse Laminin	1 mg	23017-015



Item	Quantity	Cat. no.
StemPro™ Accutase™ Cell Dissociation Reagent	100 mL	A11105-01
β-Mercaptoethanol (1,000X), liquid	50 mL	21985-023

The products listed in this section may be used with Rat Glial Precursor Cells (GPCs). For more information, refer to our website.

Item	Quantity	Cat. no.
Trypan Blue Stain	100 mL	15250-061
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
Water, distilled	20 × 100 mL	15230-196

Products for marker analysis

The products listed below may be used for analyzing the phenotype of undifferentiated Rat Glial Precursor Cells (GPCs), as well as neurons, oligodendrocytes, and 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.
Mouse anti-MAP2	100 µg	13-1500
Rabbit anti-Doublecortin (DCX)	100 µg	48-1200
Mouse anti-A2B5 (105)	100 µg	433110
Rabbit anti-GFAP (Glial Fibrillary Acid Protein) - concentrate	1 mL	18-0063
DAPI (4',6-diamidino-2-phenylindole, dihydrochloride)	10 mg	D1306



Item	Quantity	Cat. no.
ProLong™ Gold Antifade Reagent	10 mL	P36930
ProLong™ Gold Antifade Reagent with DAPI	10 mL	P36931



Introduction

Rat glial precursor cells (GPCs)

Introduction

Rat Glial Precursor Cells (GPCs), also referred to as Oligodendrocyte Progenitor Cells (OPCs) or Glial Restricted Precursor Cells (GRPCs), are isolated from the cortices of newborn Sprague-Dawley™ rats at day 2 after birth (PN2). The cells are isolated under sterile conditions using dissociation and magnetic bead separation, and expanded in complete StemPro™ NSC SFM™ containing 10 ng/mL Platelet Derived Growth Factor AA Homodimer (PDGF-AA). The cells are cryopreserved at passage 2 (P2) in 90% complete StemPro™ NSC SFM™ with 2 mM GlutaMAX™-I supplement and 10 ng/mL PDGF-AA, plus 10% DMSO.

Each vial of Rat GPCs contains 1×10^6 viable cells that can be expanded in culture for at least one passage. Withdrawal of the growth factors (*i.e.*, PDGF-AA, bFGF, and EGF) from the medium allows the cells to differentiate into a mixed population of cells, a substantial portion of which consists of oligodendrocytes (Mujtaba *et al.*, 1999). Because of their capacity to generate oligodendrocytes, Rat GPCs can be used for neuroscience studies as well as stem cell differentiation, tissue engineering, cell and genetic therapy, and transplantation experiments (Gage, 2000; Kalyani & Rao, 1998; Rao, 1999; Temple, 2001). The ability of GPCs to migrate over large distances in intact brain tissue also make these cells the ideal candidate for delivering drugs or genes in a variety of ganglioside disorders (Rao, 1999).

For optimal growth and recovery of your Rat GPCs, we recommend that you use complete GPC growth medium, which consists of complete StemPro™ NSC SFM™ supplemented with 2 mM GlutaMAX™-I and 10 ng/mL PDGF-AA (see “Preparing growth medium” on page 12 for recipe).

Characteristics of rat GPCs

- Isolated from the cortices of newborn Sprague-Dawley™ rats on postnatal day 2 (PN 2)
- Able to differentiate into oligodendrocytes
- Stain $\geq 80\%$ positive for the glial restricted precursor cell specific marker, A2B5
- Stain $\leq 10\%$ positive for differentiated phenotype markers MAP2 and GFAP
- Stain positive for the oligodendrocyte-specific marker, GalC ($\geq 30\%$) when induced to differentiate along the oligodendrocyte lineage
- Exhibit a doubling time of approximately 4 days
- Can be expanded in culture for at least one passage without differentiation



Rat GPC culture

Primary cells isolated from the cortex of newborn (postnatal day 2) Sprague-Dawley[™] rat can be expanded for at least one passage in culture upon recovery. The image below shows undifferentiated Rat GPCs at day three after thawing.

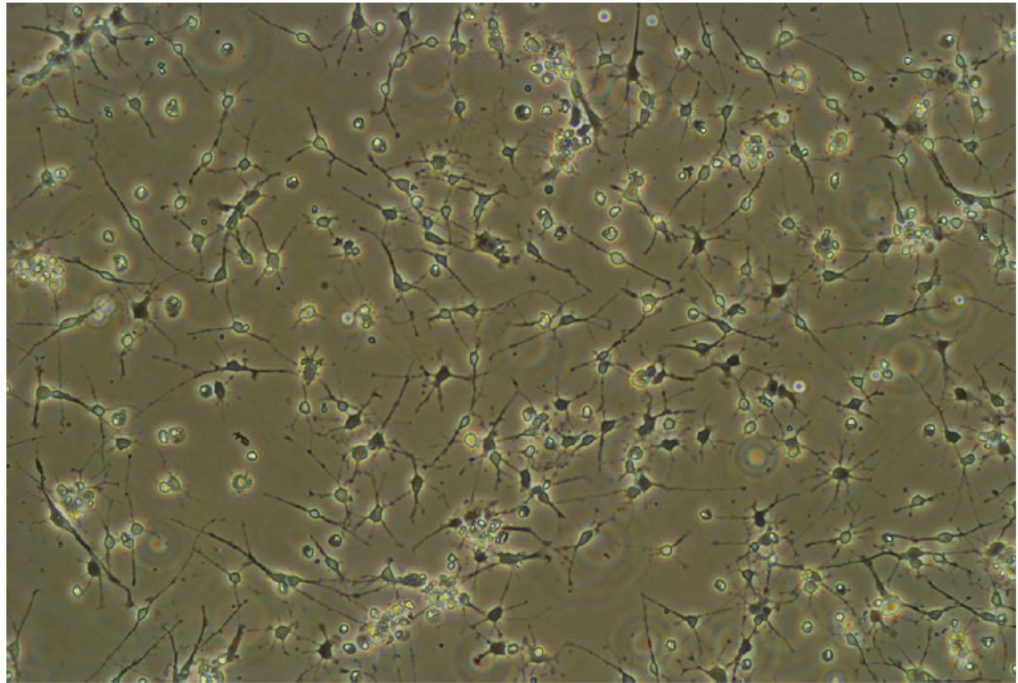


Figure 1 Bright field image of Rat GPCs at passage 3 (P3) that have been cultured in complete StemPro[™] NSC SFM[™] supplemented with 2 mM GlutaMAX[™]-I and 10 ng/mL PDGF-AA (*i.e.*, complete GPC growth medium) for three days. The image was captured using 10X objective lens.



Handling rat GPCs



CAUTION! As with other mammalian cell lines, when working with Rat GPCs, 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 GPCs

Follow the general guidelines below to grow and maintain Rat GPCs.

- **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 differentiation studies and other experiments, we recommend using cells below passage 4 (P4). If you expand Rat GPCs beyond P3 (*i.e.*, more than a single passage upon recovery), we recommend that you perform another round of characterization prior to further experiments.
- To keep Rat GPCs undifferentiated, change medium every two days.
- For general maintenance of Rat GPCs in culture, the growth rate should be in mid-logarithmic phase with 70–80% confluency prior to subculturing. Passage cells at a seeding density of 30,000 cells/cm².

Note: Passaging Rat GPCs at a lower density causes the cells to differentiate.

- When thawing or subculturing cells, transfer cells into pre-warmed medium.
- Standard physical growth conditions for Rat GPCs are 37°C in a humidified atmosphere of 5% CO₂ in air.

IMPORTANT! We recommend that you use Rat GPCs right after recovery. After thawing Rat GPCs, expand the cells once to have a two-fold increase in their number, and harvest them to use in your experiments (*e.g.*, transplantation studies, testing differentiation media).

Media requirements

IMPORTANT! It is very important to strictly follow the guidelines for culturing Rat GPCs in this manual to keep them undifferentiated.

Media requirements

We recommend using complete GPC growth medium, which consists of complete StemPro™ NSC SFM™ supplemented with 2 mM GlutaMAX™-I and 10 ng/mL PDGF-AA, for optimal growth and expansion of Rat GPCs, and to keep them undifferentiated (for a recipe, see “Preparing growth medium” on page 12). This medium is designed to support isolation and growth of glial precursor cells derived from the cortical tissue of newborn rats, which can be grown as an adherent culture on CELLStart™ or poly-L-ornithine coated tissue culture treated vessels (see “Additional products” on page 6 for ordering information).

- Prepare your growth medium prior to use.
- To maintain undifferentiated GPCs, change medium every two days.
- When thawing or subculturing cells, transfer cells into pre-warmed medium at 37°C.
- We recommend that you aliquot complete GPC growth medium into required working amounts to avoid exposing it to 37°C multiple times. Complete GPC growth medium is stable for up to two weeks when stored **in the dark** at 4°C. Do **not** freeze complete GPC growth medium.
- You may store the complete StemPro™ NSC SFM™ **in the dark** at 4°C for up to four weeks. Do **not** freeze complete StemPro™ NSC SFM™.
- You may refreeze unused StemPro™ NSC SFM™ Supplement; however, avoid repeated freeze-thaw cycles.

Preparing growth medium

Preparing growth medium

Complete GPC growth medium consists of complete StemPro™ NSC SFM™ (KnockOut™ D-MEM™/F-12 with StemPro™ NSC SFM™ Supplement, EGF, and bFGF) supplemented with 2 mM GlutaMAX™-I and 10 ng/mL PDGF-AA.

Complete GPC growth medium is stable for up to two weeks when stored **in the dark** at 4°C.

To make 100 mL of complete GPC growth medium, aseptically mix the following components:

Component	Concentration	Amount
KnockOut™ D-MEM™/F-12	1X	97 mL
GlutaMAX™-I Supplement	2 mM	1 mL
bFGF	20 ng/mL	2 µg
EGF	20 ng/mL	2 µg



Component	Concentration	Amount
StemPro™ NSC SFM™ Supplement	2%	2 mL
PDGF-AA	10 ng/mL	1 µg

Note: You may observe a white precipitate when thawing StemPro™ NSC SFM™ supplement, which will disappear when it is completely thawed or dissolved.

Preparing Matrix™ for adherent cell culture

Matrix™ for rat GPC culture

You may use CELLStart™ or poly-L-ornithine as a matrix for maintaining your Rat GPCs. The attachment strength of Rat GPCs may be greater for CELLStart™ than poly-L-ornithine, depending upon the tissue culture vessel used.

Materials needed

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

- Tissue-culture treated flasks, plates, or Petri dishes
- CELLStart™ or poly-L-ornithine (Sigma, Cat. no. P3655)
- D-PBS containing calcium and magnesium, but no phenol red
- Cell culture grade, distilled water (if coating plates with poly-L-ornithine)
- 37°C incubator with a humidified atmosphere of 5% CO₂
- *Optional:* Parafilm™ (if planning to store coated dishes for future use)

Coating culture vessels with CELLStart™

Do not freeze, vortex, or expose CELLStart™ to vigorous agitation to prevent potential gel formation. You may coat the plates in advance, and store at 4°C wrapped tightly with Parafilm™ for up to 2 weeks. **Do not** remove CELLStart™ solution until just prior to use. Make sure the plates **do not** dry out.

1. Dilute CELLStart™ 1:100 in D-PBS **with** calcium and magnesium to prepare the working solution of CELLStart™ (*i.e.*, 50 µL of CELLStart™ into 5 mL of D-PBS).
2. Coat the surface of the culture vessel with the working solution of CELLStart™ (14 mL for T75, 7 mL for T25, 3.5 mL for 60-mm dish, 2 mL for 35-mm dish).
3. Incubate the culture vessel at 37°C in a humidified atmosphere of 5% CO₂ in air for 1 hour.
4. Remove the vessel from the incubator and store until use. Immediately before use, aspirate all CELLStart™ solution and replace with complete growth medium (see “Preparing growth medium” on page 12).

Note: You do **not** need to wash the culture dishes coated with CELLStart™; you may use them directly after aspiration.



Coating culture vessels with Poly-L-ornithine

You may coat the plates with poly-L-ornithine in advance, and store at room temperature wrapped tightly with Parafilm™ for up to 1 week. **Do not** remove D-PBS until just prior to use. Make sure the plates **do not** dry out.

1. Dissolve poly-L-ornithine (Sigma, Cat. no. P3655) in distilled water to make 10 mg/mL stock solution (500X). Aliquot and store at -20°C until use.
2. Dilute poly-L-ornithine stock solution 1:500 in cell culture grade distilled water make 20 $\mu\text{g}/\text{mL}$ working solution.
3. Coat the surface of the culture vessel (with or without cover slips) with poly-L-ornithine working solution (14 mL for T75, 7 mL for T25, 3.5 mL for 60-mm dish, 2 mL for 35-mm dish).
4. Incubate the culture vessel overnight at room temperature.
5. Rinse the culture vessel twice with D-PBS **without** Ca^{2+} and Mg^{2+} , and store covered with D-PBS until use. Immediately before use, remove all D-PBS and replace with complete growth medium (see “Preparing growth medium” on page 12).

Thawing and establishing cells

Materials Needed

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

- Rat GPCs, stored in liquid nitrogen
- Ethanol or 70% isopropanol
- Complete growth medium (complete GPC growth medium, see “Preparing growth medium” on page 12); 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
- CELLStart™ or poly-L-ornithine coated, tissue-culture treated flasks, plates, or Petri dishes (see “Coating culture vessels with CELLStart™” on page 13)
- 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 “Products for marker analysis” on page 7 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 GPCs 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 GPCs, follow the procedure on the next page.

Thawing procedure

1. Pre-rinse your culture flasks, plates, or Petri dishes with growth medium to coat the plastic surface. Make sure to pre-rinse any other material that will come in contact with the cells to prevent cells from sticking to the plastic.
2. Remove the cells from liquid nitrogen storage, and **immediately** transfer the cells to a 37°C water bath to prevent crystal formation.
3. Quickly thaw the vial of cells by gently swirling it in the 37°C 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.
4. When thawed, transfer the tube containing the cells into the laminar flow hood, and wash the outside of the tube with 70% isopropanol.
5. Rinse the pipette tip with media, and very gently transfer the cells into a pre-rinsed 15-mL centrifuge tube.
6. Rinse the vial with 1 mL of growth medium (see “Preparing growth medium” on page 12), and dropwise add to the cells in the 15-mL centrifuge (one drop/second). Mix by gentle swirling after each drop.
7. Slowly add 2 mL of growth medium to the cell solution, and mix gently.
8. Determine the viable cell count using your method of choice. The viability of thawed cells should be >50%, and the total live cell number should be $>1 \times 10^6$.
9. Plate the cells at a seeding density of 3×10^4 – 5×10^4 cells per cm^2 on a CELLStart™ or poly-L-ornithine coated, tissue-culture treated culture dish. If necessary, gently add growth medium to the cells to achieve the desired cell concentration and recount the cells.
10. Incubate at 37°C, 5% CO₂, and 90% humidity and allow cells to adhere for at least 24 hours.
11. The next day, replace the medium with an equal volume of fresh, pre-warmed complete growth medium. Change the medium every other day, and passage cells when the culture is 75–90% confluent.



Expanding cells

Introduction

You may expand Rat GPCs as an adherent culture on CELLStart™ or poly-L-ornithine coated, tissue-culture treated flasks, plates or dishes. Harvest your cells when 70–80% confluent, before colonies start contacting each other.

Note: We recommend that you use Rat GPCs right after recovery. Upon thawing Rat GPCs, expand the cells **once** to have a two-fold increase in their number, and harvest them to use in your experiments (e.g., transplantation studies, testing differentiation media).

Materials needed

The following materials are required for passaging Rat GPCs (“Additional products” on page 6 for ordering information).

- Culture vessels containing Rat GPCs (70–80% confluent)
- CELLStart™ or poly-L-ornithine coated, tissue-culture treated flasks, plates, or Petri dishes (see “Coating culture vessels with CELLStart™” on page 13)
- Complete growth medium (see “Preparing growth medium” on page 12), pre-warmed to 37°C
- Disposable, sterile 15-mL conical tubes, pre-rinsed with medium
- 37°C incubator with humidified atmosphere of 5% CO₂
- Dulbecco’s Phosphate Buffered Saline (D-PBS), containing no calcium, magnesium, or phenol red
- StemPro™ Accutase™ Cell Dissociation Reagent, pre-warmed to 37°C
- Hemacytometer, cell counter and Trypan Blue, LIVE/DEAD™ Cell Vitality Assay Kit, or the Countess™ Automated Cell Counter

IMPORTANT! Rat GPCs 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 expand Rat GPCs, follow the procedure on the next page.

Harvesting rat GPCs

1. Remove the spent growth medium from the culture dish containing the cells, and store in a 15-mL tube to use as a washing solution.
2. Rinse the surface of the cell layer once with D-PBS without Ca²⁺ and Mg²⁺ (approximately 2 mL D-PBS per 10 cm² 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. Once you observe cell detachment, gently pipette up and down to break clumps into a single cell suspension. Stop the cell dissociation reaction by adding 7 mL of the spent medium from step 1 on page 16. Disperse the medium by pipetting over the cell layer surface several times
6. Transfer the cells to a new 15-mL conical tube, pre-rinsed with medium, and centrifuge at $300 \times g$ for 7 minutes at room temperature. Aspirate and discard the medium.
7. Resuspend the cell pellet in a minimal volume of pre-warmed complete growth medium and remove a sample for counting.
8. Determine the total number of cells and percent viability using your method of choice. If necessary, add complete growth medium to the cells to achieve the desired cell concentration and recount the cells.
9. Add complete growth medium to the tube containing cells so that the final viable cell concentration is 1×10^4 cells per μL .
10. Add the appropriate volume of cells to each CELLStart™ or poly-L-ornithine coated flask, plate, or Petri dish to the correct seeding density of 3×10^4 cells per cm^2 .
11. Incubate cells at 37°C , 5% CO_2 , and 90% humidity, and change growth medium every other day.

Differentiating rat GPCs

Introduction

One critical hallmark of GPCs is their capacity to differentiate into oligodendrocytes (Rao, 1999). Traditional and modern bioassays are used to demonstrate the ability of Rat GPCs to differentiate along this lineage. This section provides guidelines for spontaneously differentiating Rat GPCs.

Note: Spontaneous differentiation of Rat GPCs will result in a mixed population of cells; however, a large proportion of the differentiated cells will be oligodendrocytes.

Materials needed

In addition to materials for expanding Rat GPCs (see “Materials needed” on page 16), the following materials are required:

- Complete growth medium (complete GPC growth medium) without PDGF-AA, bFGF, or EGF (*i.e.*, complete StemPro™ NSC SFM™ supplemented with 2 mM GlutaMAX™-I, but without PDGF-AA, bFGF, or EGF) and supplemented with 2% Fetal Bovine Serum (FBS)
- Poly-L-ornithine **and** laminin coated (*i.e.*, double-coated), tissue-culture treated plate



Spontaneous differentiation protocol

To spontaneously differentiate Rat GPCs into oligodendrocytes, follow the protocol below.

1. Plate Rat GPCs on a poly-L-ornithine **and** laminin coated, tissue culture-treated plate at 3×10^4 cells/cm² following the protocol for expanding Rat GPCs (see “Harvesting rat GPCs” on page 16).
2. After 2 days, change medium to complete StemPro™ NSC SFM™ supplemented with 2 mM GlutaMAX™-I and 2% FBS, but without PGDF-AA, bFGF, or EGF (*i.e.*, withdraw growth factors from cell culture), and replace with fresh medium every other day.

Characterizing phenotype of rat GPCs

Introduction

This section provides information on phenotypic marker expression of Rat GPCs in their undifferentiated state, and after their differentiation into oligodendrocytes.

Phenotypic markers

The following table lists the primary antibodies used for classifying undifferentiated Rat GPCs as well as oligodendrocytes, neurons, and 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
Undifferentiated GPCs	A2B5 (Invitrogen™, Cat. no. 433110)	1:200	Mouse IgG
Neurons	MAP2 (Invitrogen™, Cat. no. 13-1500)	1:200	IgG1, kappa
	DCX (Invitrogen™, Cat. no. 48-1200)	1:400	Rabbit IgG
Oligodendrocytes	GalC (Millipore™, Cat. no. MAB342)	1:200	Mouse IgG
Astrocytes	GFAP (Invitrogen™, Cat. no. 18-0063)	1:200	Rabbit IgG



See Figure 2 and Figure 3 for examples of fluorescent images showing phenotypic marker expression of Rat GPCs in their undifferentiated state, and after their differentiation into oligodendrocytes.

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 25 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°C. **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 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 GPCs

Undifferentiated rat GPCs

The presence of Platelet Derived Growth Factor AA Homodimer (PDGF-AA), basic Fibroblast Growth Factor (bFGF), and Epidermal Growth Factor (EGF) in the complete GPC growth medium allows the Rat GPCs to remain undifferentiated. The images below show the phenotype marker expression of undifferentiated Rat GPCs cultured in complete GPC growth medium for three days.

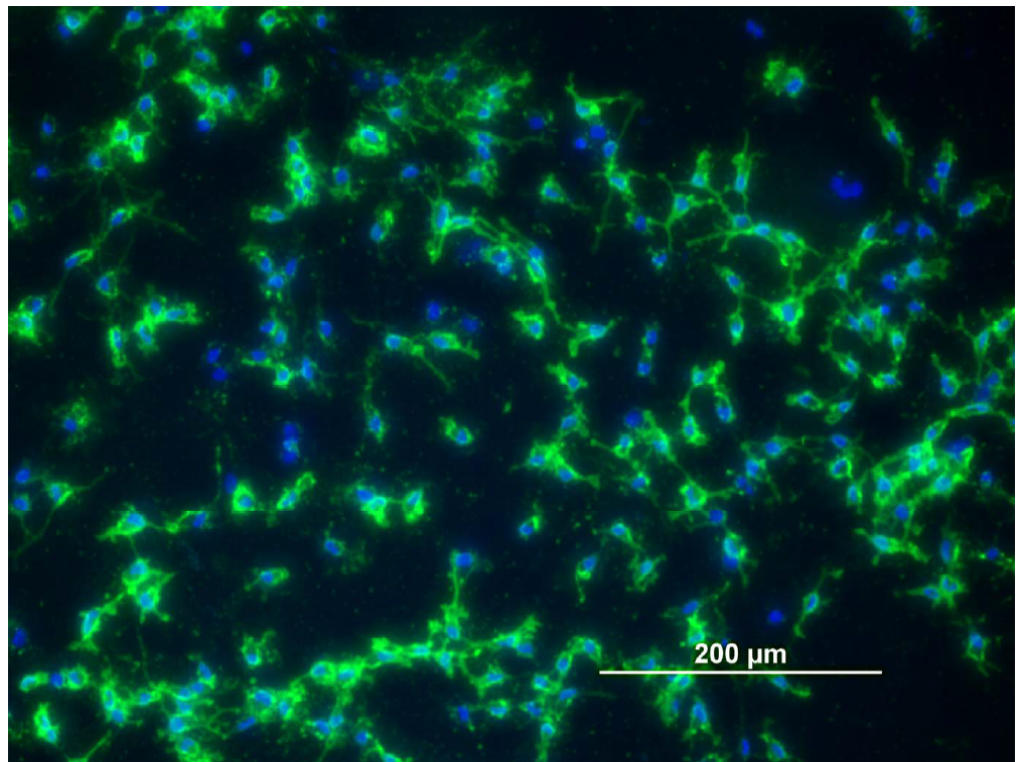


Figure 2 Rat GPCs stained by indirect immunofluorescence for the cell-surface marker A2B5 (green) showing $\geq 80\%$ expression. Nuclei were stained with DAPI (blue). The cells were maintained in the undifferentiated state in complete GPC growth medium (complete StemPro™ NSC SFM™ supplemented with 10 ng/mL PDGF-AA) for three days prior to 4% paraformaldehyde fixation and staining. Rat GPCs from the same culture showed $\leq 10\%$ positive staining for the differentiated lineage markers GFAP and MAP2 (data not shown). Scale bar = 200 μm .



Differentiated rat GPCs

Rat GPCs spontaneously differentiate upon withdrawal of growth factors from culture media, and the addition of 2% FBS. The images below show the phenotype marker expression of Rat GPCs after they were cultured in GPC growth medium, and allowed to differentiate spontaneously.

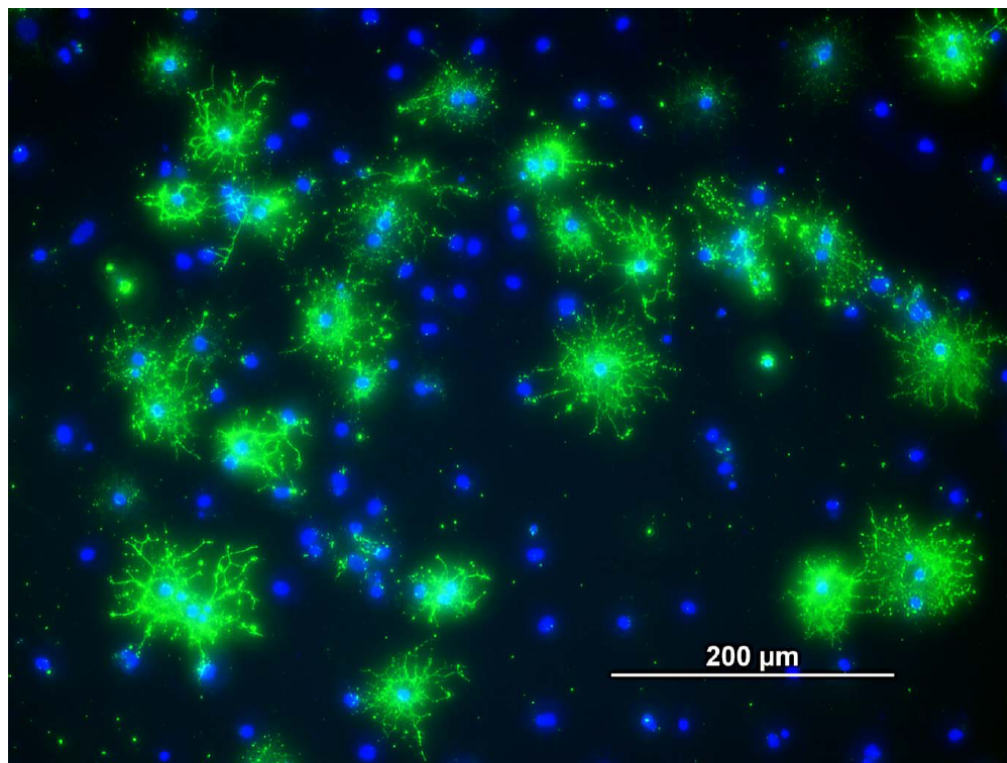


Figure 3 Rat GPCs differentiating to oligodendrocytes. The cells were differentiated in growth medium supplemented with 2% FBS and lacking PDGF-AA, bFGF, and EGF for three days prior to fixation with 4% paraformaldehyde and subsequent staining. The cell-surface marker GalC was detected by indirect immunofluorescence (green) in $\geq 30\%$ of the cells in the culture. Nuclei were stained with DAPI (blue). 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.
	Home-made stock not viable	Freeze cells at a density of 1×10^6 viable cells/mL.
		Use low-passage cells to make your own stocks.
		Follow procedures in Thawing and Establishing Cells (“Thawing and establishing cells” on page 14) 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°C water bath.
		Obtain new Rat GPCs.
	Thawing medium not correct	Use pre-warmed complete growth medium, prepared as described in “Preparing growth medium” on page 12.
	Cells too diluted	Generally we recommend a culture density of 3×10^4 – 5×10^4 cells per cm^2 at the time of recovery.
	Cell not handled gently	Rat GPCs are fragile; treat your cells gently, do not vortex, bang the flasks to dislodge the cells, or centrifuge the cells at high speeds.



No viable cells after thawing stock	Poly-L-ornithine incompletely removed from culture vessel	Poly-L-ornithine is toxic to cells. Completely remove poly-L-ornithine from the culture vessel by washing the vessel twice with PBS without Ca^{2+} and Mg^{2+} .
Fewer viable cells than expected after thawing stock	Cells sticking to plastic culture vessel	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	Growth medium not correct	Use pre-warmed complete growth medium (see “Preparing growth medium” on page 12).
	Cells have been passaged more than once.	Obtain new, P2 Rat GPCs.

Culturing cells

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

Problem	Cause	Solution
Cells differentiated	Culture conditions not correct	Thaw and culture fresh vial of new Rat GPCs. Follow thawing instructions (“Thawing and establishing cells” on page 14) and expansion procedures (“Expanding cells” on page 16) exactly. Do not omit PDGF-AA from the medium.
	Cell seeding density at the time of plating is too low or too high	Cells passaged too sparsely or cells allowed to get too confluent can cause differentiation. Seed cells at a density of 3×10^4 – 5×10^4 cells per cm^2 .



Problem	Cause	Solution
Cells differentiated	Growth medium does not contain PDGF-AA, bFGF, or EGF	Obtain new, P2 Rat GPCs, and do not omit PDGF-AA, bFGF, or EGF from the growth medium.
Cells not adherent after initial thaw	Used D-PBS without Ca^{2+} and Mg^{2+} for CELLStart™	Be sure to prepare CELLStart™-coated culture vessels using D-PBS containing Ca^{2+} and Mg^{2+} (see “Additional products” on page 6 for ordering information).
	CELLStart™ too dilute	You may increase the concentration of CELLStart™ up to 1:50 for better adhesion.
	Incubation for poly-L-ornithine too short	Make sure you incubate your culture vessel overnight at room temperature after coating it with poly-L-ornithine.

Differentiating cells

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

Problem	Cause	Solution
Cells fail to differentiate	Culture medium contains PDGF-AA, bFGF, or EGF	Remove PDGF-AA, bFGF, and EGF from culture medium.
	Cell density too high, and endogenous PDGF-AA and bFGF are preventing differentiation	Reduce cell density.
	Cells have been passaged more than once	Obtain new, P2 Rat GPCs.
Differentiating oligodendrocytes detach from surface or die	Cells are not sufficiently attached to the culture vessel	Use culture vessels double-coated with poly-L-ornithine and laminin.



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

- Gage, F. H. (2000) Mammalian neural stem cells. *Science* 287, 1433-1438
- Kalyani, A. J., and Rao, M. S. (1998) Cell lineage in the developing neural tube. *Biochem Cell Biol* 76, 1051-1068
- Mujtaba, T., Piper, D. R., Kalyani, A., Groves, A. K., Lucero, M. T., and Rao, M. S. (1999) Lineage-restricted neural precursors can be isolated from both the mouse neural tube and cultured ES cells. *Dev Biol* 214, 113-127
- Rao, M. S. (1999) Multipotent and restricted precursors in the central nervous system. *Anat Rec* 257, 137-148
- Temple, S. (2001) The development of neural stem cells. *Nature* 414, 112-117



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

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