

Spontaneous Differentiation of Rat Glial Precursor Cells (GPCs)

One critical hallmark of Glial Precursor Cells (GPCs) is their capacity to differentiate into oligodendrocytes. Follow the guidelines below 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

- Culture vessels containing Rat GPCs (Cat. no. N7746-100), 70–80% confluent
- CELLStart™ (Cat. no. 10142-01), fibronectin (Cat. no. 33016-015), or poly-L-ornithine coated, tissue-culture treated flasks, plates, or Petri dishes
- Complete GPC growth medium*, pre-warmed to 37°C
*Complete StemPro® NSC SFM (Cat. no. A1050901) supplemented with 2 mM GlutaMAX™-I (Cat. no. 35050-061) and 10 ng/mL PDGF-AA (Cat. no. PHG0035)
- Complete GPC growth medium without PDGF-AA, bFGF, or EGF, and supplemented with 2% Fetal Bovine Serum (Cat. no. 16141-061)
- Poly-L-ornithine **and** laminin (Cat. no. 23017-015) coated (*i.e.*, double-coated), tissue-culture treated plate
- Disposable, sterile 15-mL or 50-mL conical tubes, pre-rinsed with medium
- 37°C incubator with humidified atmosphere of 5% CO₂
- Dulbecco's Phosphate Buffered Saline (D-PBS) without Ca²⁺, Mg²⁺, or phenol red (Cat. no. 14190-144)
- StemPro® Accutase® (Cat. no. A11105-01), pre-warmed to 37°C
- Hemacytometer, cell counter and Trypan Blue (Cat. no. 15250-061), LIVE/DEAD® Cell Vitality Assay Kit (Cat. no. L34951), or the Countess™ Automated Cell Counter (Cat. no. C10227)

Spontaneous Differentiation Protocol

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.
2. After 2 days, change medium to complete StemPro® NSC SFM supplemented with 2 mM GlutaMAX™-I and 2% FBS, but without PDGF-AA, bFGF, or EGF (*i.e.*, withdraw growth factors from cell culture), and replace with fresh medium every other day.

Phenotypic Markers of Rat GPCs

The following table lists the primary antibodies used for classifying undifferentiated Rat GPCs as well as neurons, oligodendrocytes, and astrocytes.

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	IgG ₁ , 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

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