invitrogen™

Thawing and Establishing Rat Glial Precursor Cells (GPCs)

We recommend that you use Rat Glial Precursor Cells 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.

Materials Needed

- Rat Glial Precursor Cells (Cat. no. N7746-100), stored in liquid nitrogen
- CELLStart™ 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)
- Disposable, sterile 15-mL conical tubes, pre-rinsed with growth medium
- 37°C water bath
- 37°C incubator with humidified atmosphere of 5% CO_2
- Flame-polished and autoclaved glass Pasteur pipettes, or plastic Pasteur pipettes pre-rinsed with growth medium
- 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)

Protocol

Note: 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.

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, and **dropwise** add to the cells in the 15-mL centrifuge (one drop per 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% CO_2, 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.

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