Materials and Reagents

- **Rat Primary Cortical Astrocytes** (stored in liquid nitrogen)
- **Astrocyte Growth Medium**
  - 85% Dulbecco's Modified Eagle Medium (DMEM) with 4.5 g/L glucose
  - 15% Fetal Bovine Serum (FBS)
- **Dulbecco's Phosphate Buffered Saline (D-PBS)** (without calcium and magnesium)
- **Trypan Blue Stain** (for viability assessment)
- **Sterile 15 mL conical tubes**
- **Trypsin-EDTA (0.05%)**
- **Hemacytometer or Automated Cell Counter**
- **Tissue-culture treated flasks, plates, or Petri dishes (uncoated)**
- **37°C Water Bath**
- **37°C Incubator with 5% CO_2 and 90% Humidity**

Thawing Rat Primary Cortical Astrocytes

1. **Prepare Growth Medium:** Pre-warm Astrocyte Growth Medium to **37°C**.
2. **Thaw Cells**
   - Remove the vial from liquid nitrogen.
   - Quickly thaw in a **37°C water bath**, gently swirling the vial.
   - Do not submerge the cap; thaw within **<2 minutes**.
3. **Transfer Cells**
   - Transfer the cells into a **15 mL tube**.
   - Rinse the vial with **1 mL of growth medium** and add dropwise to the cells.
   - Slowly add **8 mL of growth medium**, swirling gently.
4. **Centrifugation:** Spin at **250 × g for 5 min** at room temperature.
5. **Resuspend Cells:** Aspirate the supernatant and resuspend in **2 mL of fresh growth medium**.
6. **Cell Counting:** Determine cell viability using **Trypan Blue Staining**.
7. **Plating Cells:** Seed cells at **20,000 cells/cm²** in a **pre-warmed, uncoated culture dish**.
8. **Incubation:** Place in a **37°C, 5% CO_2 incubator**.
9. **Media Change:** Replace **50% of the medium every 4–5 days**.
10. **Passage when 100% Confluent.**

Passaging Rat Primary Cortical Astrocytes

1. **Prepare Materials**
   - Warm **Astrocyte Growth Medium** and **Trypsin-EDTA** to **37°C**.
2. **Remove Spent Medium**

   - Collect spent medium in a **sterile tube** for later use.
3. **PBS Wash**
   - Rinse cells once with **D-PBS (no Ca^{2+}, Mg^{2+})** (2 mL per **10 cm^2** surface).
4. **Cell Detachment**
   - Add **3 mL of Trypsin-EDTA per T75 flask** (adjust for other dish sizes).
   - Incubate for **up to 5 minutes at 37°C**, checking for detachment.
5. **Stop Reaction**
   - Add **equal volume of collected medium** to stop the trypsin reaction.
   - Gently pipette up and down to break clumps.
6. **Centrifugation**
   - Transfer cells to a **15 mL tube**, spin at **250 × g for 5 min**.
7. **Resuspend Cells**
   - Use **pre-warmed growth medium**, mix gently, and count cells.
8. **Re-seeding**
   - Plate at **20,000 cells/cm^2** in **uncoated tissue-culture flasks**.
   - Change **medium every 4–5 days**.

Growth Conditions

- **Temperature:** 37°C
- **CO_2 Level:** 5%
- **Humidity:** 90%
