Materials and Reagents

- **SH-SY5Y Cells** (stored in liquid nitrogen)
- **Culture Medium**
  - DMEM high glucose supplemented with
  - 10% Fetal Bovine Serum (FBS)
  - 1% Penicillin-Streptomycin (PS)
- **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**
- **37°C Water Bath**
- **37°C Incubator with 5% CO_2 and 100% Humidity**

Thawing SH-SY5Y Cells

1. **Prepare Culture Medium:** Pre-warm **DMEM high glucose + 10% FBS +1% PS** 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 culture medium**.
6. **Cell Counting:** Determine cell viability using **Trypan Blue Staining**.
7. **Plating Cells:** Seed cells at **1.5 million cells per 35 mm Petri dish**
8. **Incubation:** Place in a **37°C, 5% CO_2 incubator**.
9. **Media Change:** Replace **50% of the medium every 2–3 days**.
10. **Passage when 80-90% confluent.**

Passaging SH-SY5Y Cells

1. **Prepare Materials**
   - Warm **culture 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 **1 mL of Trypsin-EDTA per T25 flask** (adjust for other dish sizes).
   - Incubate for **2–5 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 **1.5 million cells per 35 mm Petri dish**.
   - Change **medium every 2–3 days**.

Growth Conditions

- **Temperature:** 37°C
- **CO_2 :** 5%
- **Humidity:** 100%
