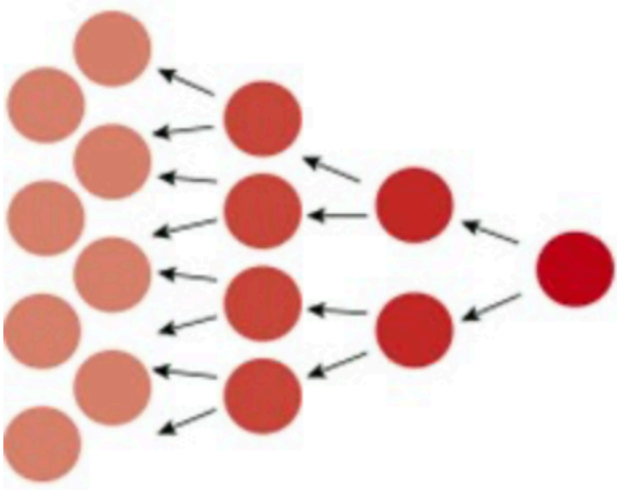
Permanently label cells with Invitrogen CellTrace fluorescent stains without affecting morphology or physiology to trace generations or divisions *in vivo* or *in vitro*.

- **Superior performance**—bright, single-peak staining enables visualization of multiple generations
- **Long-term signal stability**—well retained in cells for several days post-stain
- **Non-cytotoxic**—no known effect on proliferative ability or biology of cells
- **Versatile**—multiple colors available to easily combine with antibodies or markers of cell function, such as GFP



Materials and Reagents:

- **CFSE (CellTrace™ CFSE, Invitrogen, C34554)**
- **DMSO (Solvent for CFSE)**
- **Culture medium:** DMEM/high glucose + 10% FBS + 1% Peni/Strepto
- **PBS without calcium and magnesium (1X)**
- **HBSS with calcium and magnesium**
- **Trypsin-EDTA 0.05%**
- **35 mm culture dishes**
- **Flow cytometer (excitation 488 nm, emission 517 nm)**

Preparation of CFSE Solution :

1. Prepare a **stock solution** of CFSE at **5 mM** by dissolving **50 µg CFSE** in **18 µL DMSO**.
2. Aliquot and store at **-20°C in the dark**. **Avoid multiple freeze-thaw cycles.**
3. **Prepare the working solution (1 µM) just before use:**
    - Dilute **1:1000** the stock solution in **pre-warmed PBS at 37°C**.
    - Example: Add **1 µL of stock solution to 1 mL of PBS** to obtain a final concentration of **1 µM**.

Experimental Protocol :

1. Cell Preparation

- **Day -3:** Seed **1.5 million SH-SY5Y cells** per **35 mm Petri dish** in **2 mL of culture medium**.
- **Day 0 (Experimentation)**
    - Ensure cells are **80% confluent**.
    - Replace the culture medium with **2 mL of pre-warmed PBS 1X** and wash **once**.

2. Cell Labeling with CFSE

1. Replace PBS with **1 mL of CFSE solution at 1 µM per dish**.
2. Incubate for **20 minutes at 37°C in the dark**.
3. Stop the reaction by adding **2 mL of complete medium (DMEM 10% FCS+ 1% penicillin-streptomycin)** and incubate for **5 minutes at 37°C**.
4. Wash **twice** with culture medium by centrifuging cells (1200 rpm, 5 minutes).

3. Culture and Analysis

1. Incubate cells in **fresh? culture medium** for **10 minutes** to allow CFSE hydrolysis.
2. Perform **experimental treatments** if necessary.
3. **Flow cytometry analysis**
    - Detach cells with **500 µL trypsin per dish** and incubate for **5 minutes at 37°C**.
    - Add **1 mL of medium**, collect cells into flow cytometry tubes.
    - Wash **twice with PBS** (centrifuge 5 min, 1200 rpm).
    - Resuspend in **500 µL HBSS+** and analyze.

Flow Cytometry Parameters:

- **Excitation:** 488 nm (blue laser)
- **Emission:** 517 nm (FITC filter)
- **Compensation:** Include a tube with unstained cells to define baseline fluorescence.

Data Analysis:

1. Compare **CFSE fluorescence intensity** between treated and untreated populations.
2. Verify **cell viability** using **Sytox Blue double staining**.
3. Calculate the **Stain Index:**

   (Median Pop CFSE+ - Median Pop CFSE-) / SD pop-negative

Notes and Adjustments:

- To adjust CFSE concentration, test a range of **1-10 µM** depending on the cell type.
- In case of **high cell mortality**, reduce incubation time or CFSE concentration.
- If **fluorescence fades too quickly**, try incubating for **30 min at 4°C** after labeling.