Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

EZCell™ CFDA SE Cell Tracer Kit

09/20

(Catalog #K2057-200; 200 assays; Store at -20°C)

I. Introduction:

Carboxyfluorescein Diacetate Succinimidyl Ester (CFDA SE) is a colorless, non-fluorescent, stable cell-permeable dye. Once it enters into the cells, the intracellular esterases remove the acetate groups to generate Carboxyfluorescein Succinimidyl Ester, which then covalently binds to lysine residues or other amines and produces a bright fluorescent conjugate. The fluorescent conjugate is stable and is retained inside the cells during cell division and hence it is an ideal tool for cell tracing studies, both *in vitro* and *in vivo*. **BioVision's EZCell™ CFDA SE Cell Tracer Kit** provides an easy, rapid and sensitive way to determine cell proliferation using CFDA SE dye. The dye can be inherited by the daughter cells for up to 8 generations. Following each cell division, the fluorescence intensity is halved, which can be quantified by measuring the fluorescence intensity at Ex/Em = 492/517 nm. The assay is non-radioactive, accurate and can be used for cell tracing studies.

II. Applications:

- Tracing cell division *in vitro* and *in vivo*
- Monitoring cell proliferation
- Assessment of drugs that affect cell division

III. Sample Type:

- Adherent or suspension cells

IV. Kit Contents:

Components	K2057-200	Cap Code	Part Number
CFDA SE Assay Buffer	100 ml	NM	K2057-200-1
CFDA SE	5 vials	Green	K2057-200-2
DMSO	1 ml	Amber	K2057-200-3

V. User Supplied Reagents and Equipment:

- Tissue culture treated 6-well plate with clear bottom and lid
- Aluminum foil
- Sterile PBS
- Trypsin/0.25% EDTA
- Cell media
- Fetal bovine serum
- Flow cytometer

VI. Reagent Preparation and Storage:

Store the kit at -20°C, protected from light. Warm all the reagents to room temperature (RT) before use. Read the entire protocol before performing the assay.

- **CFDA SE Assay Buffer:** Warm to 37°C before use. Store at 4°C.
- **CFDA SE:** Add 179 µl of DMSO to one vial of CFDA SE to make 1 mM CFDA SE solution. Mix to dissolve completely and wrap the vial with aluminum foil to protect it from light. Store at -20°C for 2 months.
- **DMSO:** Warm to RT to thaw completely before use.

VII. CFDA SE Cell Tracer Protocol:

Notes:

The assay was developed using 3T3 (adherent) and Jurkat (suspension) cells and can be modified for any other cell lines. The protocol below refers to a 6-well tissue culture plate. Adjust the volume accordingly for other plate formats. The assay volume is 1 ml. Optimize the CFDA SE solution for your cell type. We suggest testing several concentrations of the CFDA SE solution to find the best conditions for your experimental design. Cell density depends on the cell type, and it may be necessary to adjust the cell numbers for optimal cell density.

1. Culture cells (3-5 x 10^5 cells /well) in a 6-well plate format in 2 ml complete cell media containing 10% FBS and incubate for 24 hr.

Note: Optional: Incubate cells in complete cell media containing 10% FBS in the presence or absence of various test compounds for 1-24 hr. This step is only required for studying the potential effects of test compound(s) on cell division.

2. Prepare 1 µM* **Cell Tracer Staining solution** by adding 40 µl of 1 mM CFDA SE solution to 40 ml PBS .

Notes:

***For short term studies:** We recommend using 0.05-5 µM Cell Tracer Staining solution.

For long term studies: We recommend using 5-10 µM Cell Tracer Staining solution.

3. For Adherent Cells:

a. Remove complete cell media and wash the cells with 1 ml PBS.
b. Add 1000 µl of 1X Cell Tracer Staining solution and incubate at 37°C for 30 min.
c. Remove the supernatant and wash the cells with 1 ml PBS once.

d. Trypsinize and collect the cells in 2 ml complete cell media containing 10% FBS. Add 1 ml cells into one well of a 6-well plate and adjust the volume to 2 ml with complete cell media containing 10% FBS. Incubate the plate at 37°C, 5% CO_2 incubator for the experimentally required time for cell tracing studies. Transfer the remaining 1 ml cells into a 1.5 ml microcentrifuge tube.
e. Centrifuge the remaining cells at 500 x g for 5 min at 4°C. Remove the supernatant and resuspend cells in 500 µl CFDA SE Assay Buffer.
f. Record the fluorescence intensity using flow cytometer in FL-1 channel.
g. For sequential cell tracing studies: Repeat Step c-f.

4. For Suspension Cells:
a. Collect cells in a 1.5 ml microcentrifuge tube. Centrifuge cells at 500 x g for 5 min and remove the supernatant.
b. Add 1000 µl of 1 x Cell Tracing Staining solution, poke a hole in the lid by using 18 G 11/2needle and incubate at 37°C for 30 min.
c. Centrifuge cells at 500 x g for 5 min at 4°C. and carefully remove the supernatant.
d. Wash cells once in 2 ml CFDA SE Assay Buffer, centrifuge at 500 x g for 5 min and carefully remove the supernatant.
e. Resuspend cells in 2 ml complete cell media containing 10% FBS. Add 1 ml cells into one well of a 6 well-plate and adjust the volume to 2 ml with complete cell media containing 10% FBS. Incubate the cells in 37°C, 5% CO_2 incubator for the experimentally required time for cell tracing studies. Transfer the remaining 1 ml cells into a 1.5 ml microcentrifuge tube.
f. Centrifuge the remaining cells at 500 x g for 5 min, remove the supernatant and resuspend cells in 1 ml CFDA SE Assay Buffer.
g. Record the fluorescence intensity using flow cytometer in FL-1 channel.
h. For sequential cell tracing studies: Repeat Step c-g.

5. Data Analysis:
Note: When analyzing the data, gate the cells of interest and exclude the cell debris and cell aggregates.

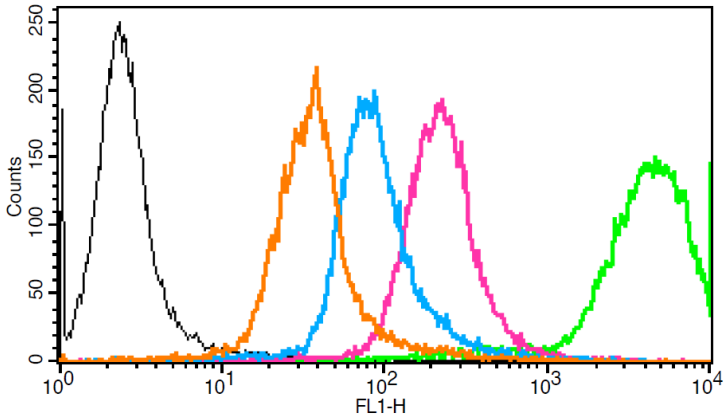

Figure: Tracking Jurkat Cell Division: Jurkat cells were seeded at $1x10^6$ cells/well into a 6-well plate in complete cell media containing 10% FBS. Cells were either stained with 0.0625 µM CFDA SE or not stained (Negative Control, black Line). Sequential cell divisions were analyzed by flow cytometry using EZCell™ CFDA SE Cell Tracer Kit. Fluorescence intensity was detected and recorded on a BD flow cytometer in FL-1 channel after staining with CFDA SE: 0 hr. (Green line); 24 hr. (Pink line); 48 hr. (Blue line) and 72 hr. (Orange line).

VIII. Related Products:

EZCell™ Cell Cycle Analysis Kit (K920)
EZCell™ Glutathione Detection Kit (K504)
Brdu Cell Proliferation Assay Kit (K306)
Quick cell Proliferation Colorimetric Assay Kit (K301)
EZCell™ Invasion Assay (K917)
EZCell™ Migration/Chemotaxic Assay Kit (K908)
EZCell™ Phagocytosis Assay Kit (K397)
Annexin V-FITC Apoptosis Detection Kit (K201)
Annexin V-Cy3 Apoptosis Kit (K102)
EZCell™ Intracellular Nitric Oxide Syhthase (NOS) Kit (K207)
EZCell™ Direct Glucose Uptake Assay Kit (K924)
EZClick™ TUNEL-in situ DNA Fragmentation/Apoptosis Kit (K191)

FOR RESEARCH USE ONLY! Not to be used on humans.