GENTAUR

DATA SHEET

Product Information

CFDA SE Cell Proliferation Kit

Catalog Number: 30050

Packaging Size: 10 X 50 ug

Molecular Weight: 557

Color and Form: White to light yellow powder

Spectral Property: $\lambda_{abs}/\lambda_{em}$ = 495/519 nm (of hydrolyzed product at neutral pH)

Kit Components:
CFDA SE: 10 vials X 50 ug lyophilized powder
DMSO: 0.5 mL anhydrous DMSO

Storage and Handling

We recommend CFDA SE vials be stored at -20°C and protected from light. The expected shelf-life under the recommended condition should be at least 6 months from the date of receipt. Working solutions of CFDA SE should be used promptly.

Product Description

The CFDA SE Cell Proliferation Kit provides convenient single-use vials for experimental studies. CFDA SE [5-(and 6)-carboxyfluorescein diacetate, succinimidyl ester] is a useful fluorescent tracer that diffuses passively into cells and covalently labels intracellular proteins, resulting in long term cell labeling. It is non-fluorescent but becomes brightly green fluorescent once it is hydrolyzed by intracellular esterases. The succinimidyl ester group reacts with intracellular amines forming fluorescent conjugates that are retained in the cell. The excess unconjugated CFDA SE diffuses passively back to the extracellular medium and can be rinsed away. The label is inherited by daughter cells through successive cell divisions. Cells labeled with CFDA SE can be subsequently fixed with formaldehyde or gluteraldehyde based fixatives.

Note: The CFDA SE dye can react with amine groups and should not be used with amine-containing buffers such as Tris-based buffers or plates and slides coated with lysine.

Protocol

The following protocol is a general labeling procedure. Because of differences in cell types and variations in culture conditions, optimization of the application is necessary. We recommend a starting concentration of 1-5 uM CFDA SE. Microscopy experiments may require up to five-fold more dye than those for flow cytometry. Use the least amount of dye as feasible to minimze adverse effects.

CFDA SE Preparation

Prepare a 5 mM CFDA SE stock solution by dissolving one 50 ug vial with 18 uL of DMSO. To prepare the working solution, dilute the stock in PBS or other non-amine containing buffer just before use.

Labeling of Cells in Suspension

1.1 Pellet cells by centrifugation and aspirate the supernatant.

1.2 Resuspend the cells in prewarmed (37°C) PBS containing CFDA SE at the appropriate concentration (working solution).

1.3 Incubate the cells for 10-15 minutes at 37°C to label the cells.

1.4 Pellet the labeled cells by centrifugation and resuspend in fresh prewarmed medium.

1.5 Incbuate the cells for an additional 15-30 minutes to ensure sufficient hydrolysis of CFDA SE.

1.6 Wash the cells once more.

1.7 Analyze by flow cytometry or microscopy.

Labeling of Adherent Cells

2.1 Grow cells to desired density on coverslips or chamber slides.

2.2 Remove the medium and add prewarmed PBS containing CFDA SE at the appropriate concentration (working solution).

1.3 Incubate the cells for 10-15 minutes at 37°C to label the cells.

1.4 Replace the labeling solution with fresh, prewarmed medium and incubate for an additional 15-30 minutes at 37°C to ensure sufficient hydrolysis of CFDA SE.

1.5 Wash the cells once more.

1.6 Analyze by microscopy using FITC filter sets.

If further processing of samples is desired, wash the cells in PBS, fix with 3.7% formaldehyde and proceed with permeabilization and staining.

References

1. Current Protocols in Cytometry, J.P. Robinson, Ed., (1998) pp 9.11.1-9.11.9