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Product Information

CFDA SE Cell Proliferation Kit

Catalog Number: 30050

Spectral Properties: $\lambda_{abs}/\lambda_{em}$ = 495/519 nm (hydrolyzed product at pH 7)

Kit Components

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

Note: Due to the small amount of material in the vial, lyophilized CFDA SE may not be visible, or it may appear as a white or yellow film on the wall of the vial.

Storage and Handling

Store lyophilized CFDA SE at -20°C and protect from light. Under these conditions the product is stable for at least 6 months from the date of receipt. Working solutions of CFDA SE should be used within one day.

Product Description

The CFDA SE Cell Proliferation Kit provides convenient single-use vials for cell labeling. CFDA SE [5-(and 6)-carboxyfluorescein diacetate, succinimidyl ester] is a 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 in viable cells. After hydrolysis, 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. After labeling with CFDA SE, cells can be fixed with formaldehyde or gluteraldehyde based fixatives.

CFDA label is inherited by daughter cells through successive cell divisions. With every cell division, each of the two daughter cells receives half of the label contained in the parent cell. Therefore, for asynchronously dividing cells, the number of cell divisions that have occurred since the cells were labeled can be tracked by flow cytometry. On a histogram of fluorescence versus counts, each cell generation will appear as a successively dimmer peak.

CFDA labeling also can be used to quantitate viable cell numbers by fluorescence microplate reader, or for uniform intracellular staining of cells for fluorescence microscopy.

Protocols

The following protocols are for use as general guidelines. Because of differences in cell types and culture conditions, optimization of the protocols is required. We recommend testing CFDA SE at a starting concentration of 1-5 uM. Microscopy experiments may require up to five-fold higher dye concentration than that used for flow cytometry. Use the lowest concentration of dye that yields good fluorescence signal to minimize cellular toxicity. For cell number quantitation, we recommend plating a standard curve of cell densities to ensure that fluorescence signal is in the linear range and is proportional to cell number. For cell division tracking, we recommend analyzing a sample of freshly labeled cells that have not allowed to divide after labeling to observe the location and intensity of the fluorescent peak representing the undivided cell population.

Note: CFDA SE dye reacts with amine groups and should not be used with amine-containing buffers such as Tris-based buffers, or with poly-lysine coated culture vessels or slides.

CFDA SE Preparation

Prepare a 5 mM CFDA SE stock solution by adding 18 uL of anhydrous DMSO to one 50 ug vial of CFDA SE. Vortex briefly to mix. To prepare the working solution, dilute the stock to the final working concentration 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 pre-warmed (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 pre-warmed cell culture medium.

1.5 Incubate the cells for at least 15-30 minutes at 37°C to ensure sufficient hydrolysis of CFDA SE.

1.6 For microplate quantitation of viable cells, proceed to step 1.7. For flow cytometry analysis of cell divisions, culture cells for the desired period of time to allow cell division to occur.

1.7 Wash the cells in PBS or other similar buffer.

1.8 Transfer cells to a multiwell plate and measure fluorescence by microplate reader, or mount cells on a slide and analyze by fluorescence microscopy. For cell division tracking, analyze by flow cytometry.

Labeling of Adherent Cells

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

2.2 Remove the medium and add pre-warmed PBS containing CFDA SE at the appropriate concentration (working solution). Use sufficient working solution to completely submerge the cells.

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

2.4 Replace the labeling solution with fresh, pre-warmed cell culture medium.

2.5 Incubate for at least 15-30 minutes at 37°C to ensure sufficient hydrolysis of CFDA SE.

2.6 For microplate quantitation or fluorescence microscopy, proceed to step 2.7. For flow cytometry tracking of cell divisions, culture cells for the desired period of time to allow cell division to occur.

2.7 Wash the cells in PBS or other similar buffer.

2.8 Analyze by fluorescent microplate reader or fluorescence microscopy. For cell division tracking, detach cells from the substrate by trypsinization or other cell dissociation method and analyze by flow cytometry.

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

Reference: Current Protocols in Cytometry. J.P. Robinson, ed. (1998). 9.11.1-9.11.9