Cell Proliferation Kit I (CFSE)

PromoKine

Catalog Number	PK-CA707-30050
Description	The PromoKine Cell Proliferation Kit I (CFSE) provides convenient single-use vials for experimental studies. CFSE, also known as CFDA-SE [5-(and 6)-carboxyfl uorescein 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. Excess unconjugated CFSE 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 CFSE 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.
Quantity	10 x 50 µg
Kit Components	CFDA-SE: 10 vials X 50 µg lyophilized powder (white to light yellow) DMSO: 0.5 mL anhydrous DMSO
Applications / Assay 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 µM CFSE. Microscopy experiments may require up to five-fold more dye than those for fl ow cytometry. Use the least amount of dye as feasible to minimze adverse effects. CFSE: MW = 557; λex/λem = 495/519 nm (hydrolyzed product at neutral pH) CFDA SE Preparation Prepare a 5 mM CFDA-SE stock solution by dissolving one 50 µg vial with 18 µL of anhydrous DMSO. Protect dye stock solutions from light. CFDA-SE dye is susceptible to hydrolysis, therefore, the DMSO stock solution should only be prepared on the day of use, and not subjected to freeze/thaw cycles. The dye should only be added to aqueous buffer immediately before staining. Do not use buffers containing Tris or other free amines. Labeling of Cells in Suspension 1.1 Pellet cells by centrifugation and aspirate the supernatant. 1.2 Resuspend the cells at 10^6 cells/ml in pre-warmed (37°C) PBS (or similar buffer) containing CFDA-SE at the appropriate concentration (working solution; recommended staining range: 1-5 µM). Protect cells from light for this and all subsequent steps. 1.3 Incubate the cells for 10-15 minutes at RT or 37°C to label the cells. 1.4 Add five-fold excess volume cell culture medium and incubate for 5 minutes at room temperature or 37°C to hydrolyze free dye. 1.5 Pellet the labeled cells by centrifugation and resuspend in fresh pre-warmed cell culture medium. 1.6 Incbuate the cells for an additional 15-30 minutes to ensure sufficient hydrolysis of CFDA-SE before analysis. Alternatively, culture cells for desired period of time to allow cells to divide. 1.7 Wash the cells in PBS once more. 1.8 **Optional**: perform formaldehyde fixation, permeabilization, and/or immunostaining. 1.9 Analyze by flow cytometry in the appropriate channel 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 sufficient volume of pre-warmed PBS containing CFDA-SE at the appropriate concentration (working solution) to completely cover cells. Protect cells from light for this and all subsequent steps. **Note**: Staining can be performed in cell culture medium containing serum, however, this results in 5-10 fold lower fluorescent signal compared to labeling in buffer without serum or other proteins. 2.3 Incubate the cells for 10-15 minutes at RT or 37°C to label the cells. 2.4 Replace the staining solution with fresh, pre-warmed medium and incubate for an additional 15-30 minutes at 37°C to ensure sufficient hydrolysis of CFDA-SE before analysis. Alternatively, culture cells for desired period of time to allow cells to divide. 2.4 Wash the cells in PBS once more.

	2.6 **Optional**: perform formaldehyde fixation, permeabilization, and/or immunostaining. 2.7 Analyze by microscopy, or harvest cells by trypsinization or other cell dissociation method for flow cytometry analysis. Analyze by flow cytometry in the appropriate channel or by microscopy using FITC filter sets.
Storage & Stability	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.

PromoCell GmbH

Sickingenstr. 63/65
69126 Heidelberg
Germany

Email: info@promokine.info
www.promokine.info

North America
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 90 93 32 (ligne verte)
Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

08/2015