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. Immediately after staining a single, bright fluorescent population will be detected by flow cytometry. Each cell division that occurs after labeling is revealed by the appearance of a successively dimmer fluorescent peak on a flow cytometry histogram (Fig. 1). Cell proliferation dyes can be used to track cell divisions *in vivo* or *in vitro*. The staining can withstand fixation and permeabilization for subsequent immunostaining. Thus, cells labeled with CFSE can be subsequently fixed with formaldehyde or gluteraldehyde based fixatives. Alternative applications of cell proliferation dyes include uniform labeling of cell cytoplasm for microscopy, or labeling cells for quantitation of cell number by microplate reader (note: detection by microplate reader can only be used to quantitate total cell number immediately after staining with cell proliferation dyes, not to track cell divisions).
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 dye concentration, staining time, and/or staining temperature may be necessary. Higher dye concentrations may be required to track more cell generations, while lower concentrations may be sufficient to track fewer divisions. We recommend using the lowest dye concentration that yields sufficient signal for your assay, because cell proliferation dyes can be toxic to cells at high concentrations. We recommend a starting concentration of 1-5 µM CFSE. 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. CFSE: MW = 557; λex/λem = 495/519 nm (hydrolyzed product at neutral pH). When used at a concentration of 1 µM in 1 ml of cells (1 x 10^6 cells/ml) each kit is sufficient for labeling up to 1000 samples. However, the exact number of assays that can be performed per kit depends on the number of cells and dye concentration used. For some, more sensitive cell types CFSE concentrations ≥5 µM might show slight toxicity. <u>CFDA SE Preparation</u> 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. <u>Labeling of Cells in Suspension</u> 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. (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.) 1.3 For labelling, incubate the cells for 10-15 minutes at RT or 37°C to allow dye uptake. 1.4 Add an equal volume of 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 an equal volume of fresh pre-warmed cell culture medium. 1.6 Incubate the cells for an additional 15-30 minutes to allow the dye to react with intracellular proteins before analysis. Alternatively, culture cells for desired period of time to allow cells to divide.

	1.7 Pellet the labeled cells by centrifugation and resuspend in an equal volume of fresh pre-warmed cell culture medium. Proceed to flow cytometry analysis (step 1.9). Alternatively, return cells to incubator and culture for the desired period of time to allow cells to divide. 1.8 **Optional**: perform formaldehyde fixation, permeabilization, and/or immunostaining. 1.9 Analyse by flow cytometry in the appropriate channel (CFSE can be detected in the FITC 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 For labeling, incubate the cells for 10-15 minutes at RT or 37°C to allow dye uptake. 2.4 Replace the staining solution with fresh, pre-warmed cell culture medium and incubate for 5 minutes at 37°C to hydrolyse free dye. 2.5 Replace that media with fresh, pre-warmed cell culture medium and incubate for 15-30 minutes at 37°C to allow the dye to react with intracellular proteins. 2.6 Replace with fresh, pre-warmed cell culture medium and proceed to analysis (step 2.8). Alternatively, culture cells for desired period of time to allow cells to divide. 2.7 **Optional**: perform formaldehyde fixation, permeabilization, and/or immunostaining. 2.8 Analyse by microscopy, or harvest cells by trypsinization or other cell dissociation method for flow cytometry analysis. Analyse by flow cytometry in the appropriate channel (CFSE can be detected in the FITC channel) or by microscopy using FITC filter sets.
Storage, Stability & 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. Ideally the 5 mM DMSO stock solution should be prepared on the day of use. Aliquots may be stored for later use, but activity may be reduced over time. The dyes should only be added to aqueous buffer immediately before staining. 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.
Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.

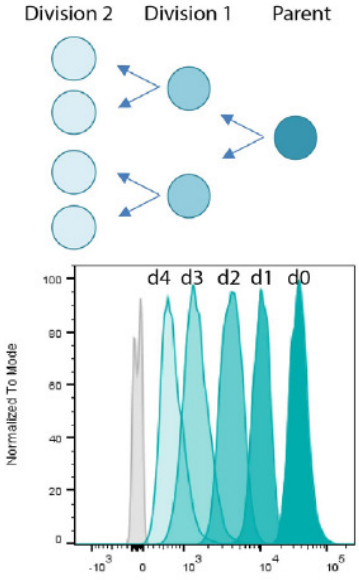

Figure 1. Principle of cell division tracking with CFSE Cell Proliferation Dye. When a stained cell divides, each daughter cell receives half the dye in the parent cell, with each cell division represented as a successively dimmer population on a flow cytometry histogram.

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