Revised: February 1, 2018

Product Information

ViaFluor® SE Cell Proliferation Kits

Kit Contents

Kit Name	Kit Cat. #	Kit Component 1: Cell Proliferation Dye	Kit Component 2: Anhydrous DMSO
ViaFluor® 405 SE Cell Proliferation Kit	30068-T	1 x 99972 (100 nmol)	99953 (150 uL)
	30068	10 x 99972 (100 nmol)	99938 (500 uL)
ViaFluor® 488 SE Cell Proliferation Kit	30086-T	1 x 99840 (100 nmol)	99953 (150 uL)
	30086	10 x 99840 (100 nmol)	99938 (500 uL)
ViaFluor® CFSE Cell Proliferation Kit	30050	10 x 99937 (90 nmol / 50 ug)	99938 (500 uL)

Kit Size

When used at a dye concentration of 1 uM in 1 mL of cells at $1x10^6$ cells/mL, each vial of ViaFluor® 405 or ViaFluor® 488 dye can be used to label 100 samples, and each vial of ViaFluor® CFSE dye can label 90 samples. The exact number of assays that can be performed per kit depends on the number of cells and dye concentration used (see Protocol and Table 1).

Storage and Handling

Store kit components at -20°C, desiccated and protected from light. Product is stable for at least 6 months from date of receipt when stored as recommended. ViaFluor® SE dyes are susceptible to hydrolysis. 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.

Spectral Properties

Dye	Abs/Em	Detection Channel
ViaFluor® 405 SE	408/452 nm	Pacific Blue®
ViaFluor® 488 SE	493/532 nm	FITC
ViaFluor® CFSE	495/519 nm	FITC

Product Description

ViaFluor® SE Cell Proliferation Dyes diffuse passively into live cells and are used for long-term cell labeling. They are initially non-fluorescent esters, but are converted to fluorescent dyes by intracellular esterases. The dyes then covalently react with amine groups on proteins, forming fluorescent conjugates that are retained in the cell. Immediately after staining a single, bright fluorescent population will be detected by flow cytometry. Each cell division that occurs after labeling results in the appearance of a 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. Staining can withstand fixation and permeabilization for subsequent immunostaining. ViaFluor® 488 is more fixable than CFSE (Fig. 2).

ViaFluor® 405 SE and ViaFluor® 488 SE Cell Proliferation dyes were developed at Biotium to provide superior cell staining, fixability, and low toxicity. ViaFluor® 405 is detected in the Pacific Blue® channel, and gives sharp peaks with no toxicity (Fig. 1). ViaFluor® 488 and ViaFluor® CFSE are both detected in the FITC channel, but ViaFluor® 488 is a less toxic, less leaky and more fixable alternative to the classic dye CFSE (Fig. 2). It also has less bleed-through into other channels such as PE.

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).

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.

Cell Proliferation Dye Preparation

Prepare a cell proliferation dye stock solution by dissolving one vial of dye in the volume of anhydrous DMSO in Table 1. Protect dye stock solutions from light. ViaFluor® SE dyes are 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 dyes should only be added to aqueous buffer immediately before staining. Do not use buffers containing Tris or other free amines.

Table 1. Preparation of ViaFluor® SE Cell Proliferation Dye stock solutions

Dye (1 vial)	Anhydrous DMSO	Stock solution concentration	Recommended staining concentration
ViaFluor® 405 SE	20 uL	5 mM	1-5 uM*
ViaFluor® 488 SE	20 uL	5 mM	1 uM*
ViaFluor® CFSE	18 uL	5 mM	1 uM*

* In our testing, ViaFluor® 405 is non-toxic to cells at 5 uM. ViaFluor® CFSE has some toxicity at 5 uM. ViaFluor® 488 has less toxicity than CFSE at 5 uM.

Labeling Cells in Suspension

1. Pellet cells by centrifugation and aspirate the supernatant.
2. Resuspend the cells at 10^6 cells/mL in pre-warmed (37°C) PBS (or similar buffer) containing 1 uM cell proliferation dye. 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.
3. Incubate the cells for 10-15 minutes at room temperature or 37°C, to allow dye uptake.
4. Add an equal volume of cell culture medium and incubate for 5 minutes at room temperature or 37°C to hydrolyze free dye.
5. Pellet the labeled cells by centrifugation and resuspend in an equal volume of fresh pre-warmed cell culture medium.
6. Incubate the cells for 15-30 minutes at 37°C to allow the dye to react with intracellular proteins.
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 9). Alternatively, return cells to incubator and culture for the desired period of time to allow cells to divide.
8. Optional: perform formaldehyde fixation, permeabilization, and/or immunostaining.
9. Analyze by flow cytometry in the appropriate channel (see Spectral Properties).

Labeling of Adherent Cells

1. Grow cells to desired density on coverslips or chamber slides.
2. Remove the medium and add a sufficient volume of pre-warmed PBS containing cell proliferation dye to completely cover cells. Protect cells from light at 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.
3. Incubate the cells for 10-15 minutes at room temperature or 37°C to allow dye uptake.
4. Replace the staining solution with fresh, pre-warmed cell culture medium and incubate for 5 minutes at 37°C to hydrolyze free dye.
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.
6. Replace with fresh, pre-warmed cell culture medium and proceed to analysis (step 8). Alternatively, culture cells for desired period of time to allow cells to divide.
7. Optional: perform formaldehyde fixation, permeabilization, and/or immunostaining.
8. Analyze by microscopy, or harvest cells by trypsinization or other cell dissociation method for flow cytometry analysis. Analyze fluorescence in the appropriate channel (see Spectral Properties).

Reference

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

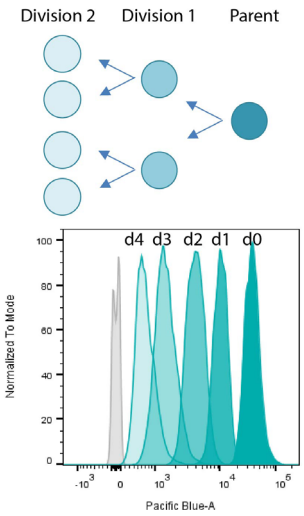

Figure 1. Principle of cell division tracking with ViaFluor® Cell Proliferation Dyes. When a stained cell divides, each daughter cell receive half the dye in the parent cell, with each cell division represented as a successively dimmer population on a flow cytometry histogram. Data shown using 5 uM ViaFluor® 405 to stain Jurkat cells.

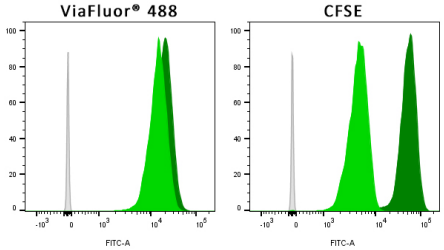

Figure 2. Figure showing improved fixability of ViaFluor® 488 over CFSE. Jurkat cells were stained with 1 uM ViaFluor® 488 (left panel) or ViaFluor® CFSE (right panel). Half of the cells were removed and subject to fixation and permeabilization (light green histogram). Cells stained with ViaFluor® 488 did not lose signal after fixation, but cells stained with ViaFluor® CFSE lost a significant amount of signal.

Related Products

Catalog No.	Product Name
32002	Live-or-Dye™ 350/448 Fixable Viability Staining Kit
32003	Live-or-Dye™ 405/452 Fixable Viability Staining Kit
32004	Live-or-Dye™ 405/545 Fixable Viability Staining Kit
32005	Live-or-Dye™ 488/515 Fixable Viability Staining Kit
32006	Live-or-Dye™ 568/583 Fixable Viability Staining Kit
32007	Live-or-Dye™ 594/614 Fixable Viability Staining Kit
32008	Live-or-Dye™ 640/662 Fixable Viability Staining Kit
32006	Live-or-Dye™ 750/777 Fixable Viability Staining Kit
32010	Live-or-Dye™ NucFix Red
22003	Mini Cell Scrapers
90082	DMSO, Anhydrous
22020	10X Phosephate-Buffered Saline (PBS)
22023	Paraformaldehyde, 4% in PBS
23006	Flow Cytometry Fixation/Permeabilization Kit
22015	Fixation Buffer
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer
22010	10% Fish Gelatin Blocking Buffer
22011	Fish Gelatin Powder
22014	30% Bovine Serum Albumin Solution
22012	Dry Milk Powder
22002	Tween®-20

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