Product Information

ViaFluor® SE Cell Proliferation Kits

Kit Contents

Kit	Cat. No.	Component 1: Proliferation Dye	Component 2: Anhydrous DMSO
ViaFluor® 405 SE Cell Proliferation Kit	30068-T	99972 1 x 100 nmol	99953 1 x 150 uL
	30068	99972 10 x 100 nmol	99938 1 x 500 uL
ViaFluor® 488 SE Cell Proliferation Kit	30086-T	99840 1 x 100 nmol	99953 1 x 150 uL
	30086	99840 10 x 100 nmol	99938 1 x 500 uL
ViaFluor® CFSE Cell Proliferation Kit	30050	99937 10 x 50 ug	99938 1 x 500 uL
ViaFluor® 650 SE Cell Proliferation Kit	30139-T	99892 1 x 100 nmol	99953 1 x 150 uL
	30139	99892 10 x 100 nmol	99938 1 x 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, ViaFluor® 488, or ViaFluor® 650 can be used to label 100 samples, and each vial of ViaFluor® CFSE 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 ViaFluor® SE Dye at -20°C, desiccated and protected from light. Store DMSO at room temperature, 4°C, or -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

Cat. No.	Dye	Ex/Em (nm)	Detection Channel
30068	ViaFluor® 405 SE	408/452	Pacific Blue®
30086	ViaFluor® 488 SE	493/532	FITC
30050	ViaFluor® CFSE	495/519	FITC
30139	ViaFluor® 650 SE	653/682	APC

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 and will covalently react with amine groups on intracellular proteins at the same time, 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 (Figure 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® 405 SE, ViaFluor® 488 SE, and ViaFluor® 650 SE were developed at Biotium to provide superior cell staining and fixability. They have been optimized to give sharp peaks with low toxicity. ViaFluor® 488 and ViaFluor® CFSE are both detected in the FITC channel, but ViaFluor® 488 is a less toxic, better retained, and more fixable alternative to the classic dye CFSE. 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, cell barcoding, or labeling cells for quantification 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 division.

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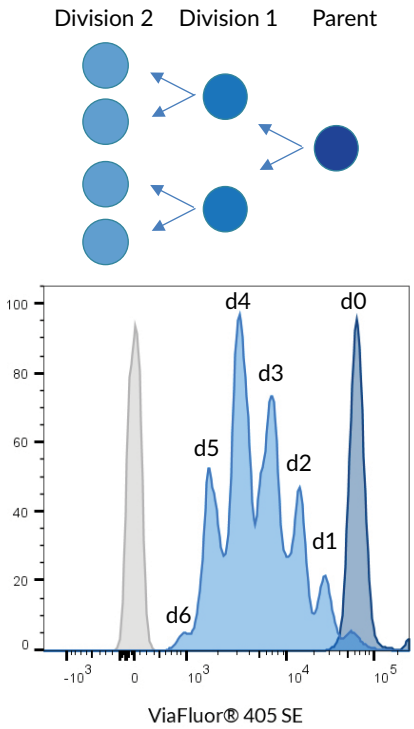


Figure 1. Principle of cell division tracking with ViaFluor® SE Cell Proliferation Dyes. When a stained cell divides, each daughter cell receives half the dye, appearing as a successively dimmer population in flow cytometry. Here, PMBCs stained with ViaFluor® 405 were stimulated with T Cell Activator Beads and IL-2, and analyzed after 4 days. T cell proliferation can be measured by the number of individual peaks (light blue). Unstimulated cells are dark blue, and unstained cells are gray.

Considerations Before Staining

ViaFluor® SE Dyes will fluoresce brightly following reaction with intracellular esterases present in live cells. However, the dyes can also stain dead cells due to residual esterase activity or hydrolysis of the AM ester by water. ViaFluor® SE Dyes are designed for monitoring cell proliferation or stable tracking of cells. They are not intended to measure cell viability. For monitoring cell viability, we recommend co-staining with a dead cell-specific DNA stain. If fixation is needed following staining, we recommend one of our Live-or-Dye™ Fixable Viability Stains (see Related Products).

Experimental 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	Anhydrous DMSO	Stock solution conc.	Recommended staining conc.
ViaFluor® 405 SE	20 uL	5 mM	1-5 uM*
ViaFluor® 488 SE	20 uL	5 mM	1-3 uM*
ViaFluor® CFSE	18 uL	5 mM	1 uM*
ViaFluor® 650 SE	20 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 cell proliferation dye (see Table 1 for recommended staining concentration). 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-fold to 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. Perform any other desired staining or other treatments (step 6) or proceed straight to flow cytometry analysis (step 7). Alternatively, return cells to incubator and culture cells for the desired period of time to allow cells to divide.
6. Optional: Perform any other desired treatments, such as immunostaining, viability staining, fixation, and/or permeabilization.
7. Analyze by flow cytometry in the appropriate channel (see Spectral Properties).

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Labeling 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 (37°C) HBSS with Ca^{2+}/Mg^{2+} containing cell proliferation dye to completely cover cells (see Table 1 for recommended staining concentration). Protect cells from light for this and all subsequent steps.

   Notes:

   a. Staining can be performed in cell culture medium containing serum, however, this results in 5-fold to 10-fold lower fluorescent signal compared to labeling in buffer without serum or other proteins.

   b. Buffer with Ca^{2+}/Mg^{2+} is usually recommended for maintaining adhesion and morphology of adherent cells.
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 with fresh, pre-warmed cell culture medium. Perform any other desired staining or other treatments (step 6) or proceed straight to analysis (step 7). Alternatively, return cells to incubator and culture cells for the desired period of time to allow cells to divide.
6. Optional: Perform any other desired treatments, such as immunostaining, viability staining, fixation, and/or permeabilization.
7. 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).

Related Products

Cat. No.	Product
90082	DMSO, Anhydrous
32002... 32018	Live-or-Dye™ Fixable Viability Staining Kits
32016	Live-or-Dye™ Fixable Viability Sampler Kit, Standard
32017	Live-or-Dye™ Fixable Viability Sampler Kit, Spectral
32010	Live-or-Dye™ NucFix™ Red
41033... 41040	NucSpot® Nuclear Stains
40085	NucSpot® Far-Red, 1000X in DMSO
40081, 40082	NucSpot® Live Cell Nuclear Stains
40060	RedDot™1 Far-Red Nuclear Stain, 200X in Water
40061	RedDot™2 Far-Red Nuclear Stain, 200X in DMSO
40037, 40084	7-AAD
40016... 40048	Propidium Iodide
40010, 40014	Ethidium Homodimer I (EthD-I)
40050, 40051	Ethidium Homodimer III (EthD-III)
30026	Calcein AM Cell Viability Assay Kit
30002	Viability/Cytotoxicity Assay for Animal Live & Dead Cells
22023	Paraformaldehyde, 4% in PBS
23006	Flow Cytometry Fixation/Permeabilization Kit
10402... 10408	NucView® Caspase-3 Enzyme Substrates
30061	CF®488A Annexin V and PI Apoptosis Kit
30060	CF®488A Annexin V and 7-AAD Apoptosis Kit
30065	Apoptosis and Necrosis Quantitation Kit Plus
30066	Apoptotic, Necrotic and Healthy Cells Quantitation Kit Plus
30072	NucView®488 & RedDot™2 Apoptosis & Necrosis Kit
30030... 30073	Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate & Annexin V
29001... 29126	Annexin V Conjugates

Please visit our website at www.biotium.com for information on our life science research products, including cell viability assays, cell and organelle stains, apoptosis reagents, fluorescent CF® Dye antibody conjugates and reactive dyes, and kits for cell biology research.

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