

Store at
-20°C

Cell Proliferation Tracer Kit (Fluorometric, Violet 450)



Cell Signaling
TECHNOLOGY®

#48444

1 Kit

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New 03/20

For Research Use Only. Not For Use In Diagnostic Procedures.

Description: The Cell Proliferation Tracer Kit (Fluorometric, Violet 450) contains Cell Proliferation Tracer Dye, Violet 450 that diffuses passively into live cells and is used for long-term cell labeling. This dye is initially a non-fluorescent ester, but is converted to a fluorescent dye by intracellular esterases. The dye then covalently reacts 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. The Cell Proliferation Tracer Kit (Fluorometric, Violet 450) can be used to track cell divisions *in vivo* or *in vitro*. Staining can withstand fixation and permeabilization for subsequent immunostaining.

Background: Due to their inherent metabolic stability once inside a cell, fluorescent proliferation dyes partition in an equal manner between daughter cells during the M phase of the cell cycle. This allows the principle of dye dilution to be leveraged as a means to trace multiple rounds of cell proliferation using flow cytometry. Added benefits of proliferation dyes are that they are non-radioactive and do not require cells to be actively synthesizing DNA for efficient uptake (1-3).

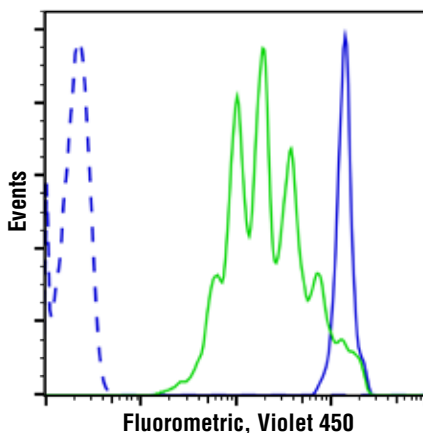
Storage: Store kit components at -20°C, desiccated and protected from light. Product is stable for at least 12 months from date of receipt when stored as recommended. Cell Proliferation Tracer dyes are susceptible to hydrolysis. Ideally, the 5 mM DMSO stock solution should be prepared on the day of use. 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.

Background References:

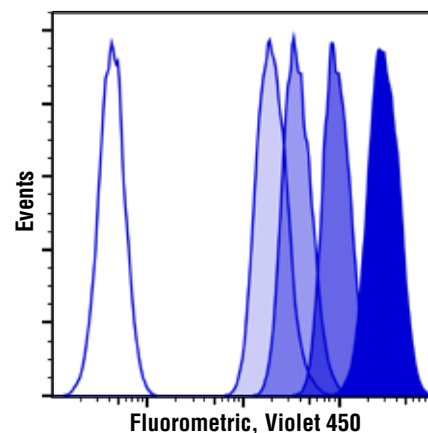
- (1) Wallace, P.K. et al. (2008) *Cytometry A* 73, 1019-34.
- (2) Quah, B.J. and Parish, C.R. (2012) *J Immunol Methods* 379, 1-14.
- (3) Tario, J.D. et al. (2011) *Methods Mol Biol* 699, 119-64.

Products Included	Product #	Quantity	Storage Temp
Cell Proliferation Tracer Dye, Violet 450	62413	1 each	-20°C
Anhydrous DMSO	15360	150 µl	-20°C

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.



Live human peripheral blood mononuclear cells were labeled with Cell Proliferation Tracer Kit (Fluorometric, Violet 450). Cells cultured in medium containing Human Interleukin-2 (hIL-2) #8907 (50 ng/mL) were then left untreated (blue solid line) or stimulated with anti-CD3 (10 µg/mL) and anti-CD28 (5 µg/mL) (green solid line). Cells were analyzed by flow cytometry after 72 hr. Unstained cells were also acquired for comparison (blue dashed line).



Cell division tracking in live Jurkat cells over the course of 4 days (d0-d3). Cells were labeled with Cell Proliferation Tracer Kit (Fluorometric, Violet 450) on day 0, and analyzed by flow cytometry each day. Each successively dimmer peak represents one cell division. Unstained cells are represented by the unshaded peak.

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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NOTE: 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.

A. Solutions and Reagents

NOTE: Cell Proliferation Tracer 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.

Supplied Reagents:

- **Cell Proliferation Tracer Dye, Violet 450 (#62413S):** Prepare a cell proliferation dye stock solution by dissolving one vial of Cell Proliferation Tracer Dye in 20 μ L of anhydrous DMSO. This brings the stock solution concentration to 5mM. Protect dye stock solutions from light.
- **Anhydrous DMSO (#15360S)**

Additional Reagents (Not Supplied):

- **1X Phosphate Buffered Saline (PBS):** To prepare 1 L 1X PBS: add 100mL 10X PBS (#12528) to 900mL water, mix.

B. Labeling of Target Cells

1. Pellet cells by centrifugation and aspirate the supernatant.
2. Resuspend cells at 10⁶ cells/mL in pre-warmed (37°C) PBS (or similar buffer) containing 1 μ M 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 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 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.