SONY

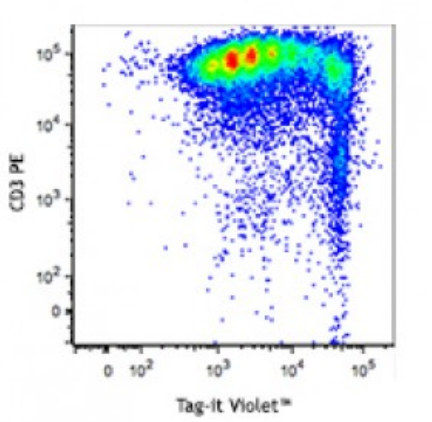
Product Data Sheet

Tag-it Violet™ Proliferation and Cell Tracking Dye

Catalog # / Size: 2725505 / 1 kit

Preparation: The Tag-it Violet™ Proliferation and Cell Tracking Dye is composed of lyophilized Tag-it Violet™ and anhydrous DMSO. For reconstitution, bring the kit to room temperature, add 50 microL of DMSO to one vial of Tag-it Violet™ dye until f

Concentration: Lot-specific



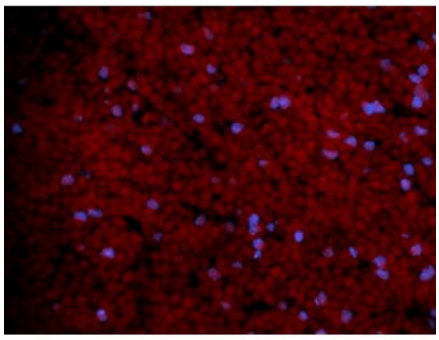
Human peripheral blood mononuclear cells were stained with Tag-it Violet™ Proliferation and Cell Tracking dye, and then stimulated with PHA for four days. On day four, cells were harvested, stained with CD3-PE and the Tag-it Violet™ signal was

Applications:

Applications: Immunofluorescence

Recommended Usage: This lot has been tested by flow cytometry for *in vitro* cell proliferation. It can be used at concentrations ranging from 1 - 20 µM for cell labeling. It is recommended that the reagent be titrated for optimal performance for each cell type, culturing condition, or application.

Application Notes: The molecular weight of Tag-it Violet™ Proliferation and Cell Tracking Dye is 489 kD. The maximum excitation and emission wavelengths of Tag-it Violet™ Proliferation and Cell Tracking Dye are 395 nm and 455 nm, respectively. Each 122.25 microg of Tag-it Violet™ Proliferation and Cell Tracking Dye may be reconstituted with 50 microL of anhydrous DMSO to yield a stock concentration of 5 mM.



Mouse spleen 72 hours after adoptive transfer of Tag-it Violet™-labeled splenocytes (purple). Nucleated cells are stained using 25 µM DRAQ™ (red). Image was captured at a 40X magnification.

Materials Provided:
5 vials x 122.25 microg Tag-it Violet™
500 microL anhydrous DMSO

Tag it-Violet™ Labeling Procedure:
1. Prior to reconstitution, spin down the

vial of lyophilized reagent in a microcentrofuge to ensure the reagent is at the bottom of the vial.
2. Prepare stock solution by reconstituting 1 vial of lyophilized Tag-it Violet™ dye in 50 microL of anhydrous DMSO to make a 5 mM solution.
3. Prepare a 5 µM working solution by diluting 1 microL of 5 mM Tag-it Violet™ stock solution in 1 mL PBS for every 1 mL of cell suspension (or at an optimal working concentration as determined by titration).
4. Spin down and resuspend cells at 10-100 x 10^6 cells/mL in the Tag-it Violet™ working solution.
5. Incubate cells for 20 minutes at room temperature or at 37°C, and keep protected from light.
6. Quench the staining by adding five times the original staining volume of cell culture medium containing 10% FBS.
7. Pellet cells and resuspend in pre-warmed cell culture medium.
8. Incubate cells for 10 minutes.
9. After incubation, Tag-it Violet™ labeled cells are ready for downstream applications or analysis9

Description: Tag-it Violet™ Proliferation and Cell Tracking Dye can be used for cell tracking and for proliferation assays. Tag-it Violet™ passively diffuses into the cell where esterases cleave acetoxymethyl esters (AM) on the molecule, Tag-it Violet™ then covalently attaches to intracellular proteins enabling its long-term retention.

Tag-it Violet™ is excited with a fluorescence wavelength of 395 nm, and emits at 455 nm. Cells labeled wtih Tag-it Violet™ can be analyzed by flow cytometry in instruments equipped with a violet laser, or visualized by fluorescence microscopy using a DAPI filter.