Violet Proliferation Dye 450

**Product Information**

**Material Number:** 562158  
**Size:** 1 mg

**Description**

BD Horizon™ Violet Proliferation Dye 450 (VPD450) is a violet laser excitable dye that can be used for flow cytometric monitoring of cell divisions completed by single cells. The nonfluorescent VPD450 dye passively diffuses across cell membranes and is cleaved by esterase activity within viable cells. The cleaved dye becomes highly fluorescent and covalently binds to protein amine groups within the cells via its succinimidyl ester group. Nonviable cells remain nonfluorescent. As viable cells divide, the VPD450 dye is distributed uniformly between daughter cells; each daughter cell retains approximately half of the VPD450 fluorescence intensity of its parent cell. The covalently bound dye is well retained within the cells allowing for applications involving live or aldehyde-fixed and permeabilized cells.

The violet laser excitable dye VPD450 emits maximally at 450 nm with minimal fluorescent spillover into the FITC channel. This allows for use with green fluorescent protein (GFP)-tagged cells and in other multicolor assays requiring measurements in the FITC channel, such as intracellular cytokine assays.

![Image of VPD450 fluorescence](Figure 1. Flow cytometric analysis of proliferative responses by human peripheral blood lymphocytes. A single-cell suspension of human peripheral blood mononuclear cells was labeled with VPD450 (1 μM) for 10 minutes. The cells were washed twice and then cultured with phytohemagglutinin for four days. The histogram shows VPD450 fluorescence peaks of gated events with the forward and side light-scatter characteristics of viable lymphocytes (successive generations of divided cells). Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.)

![Image of VPD450 staining](Figure 2. Staining of surface and intracellular phenotypes on activated mouse splenocytes. A single-cell suspension of mouse splenocytes was treated to remove red blood cells and then labeled with VPD450 (1 μM) for 10 minutes. The cells were washed twice and then cultured with purified NA/LE Hamster Anti-Mouse CD3e (Cat. No. 553057) and Purified NA/LE Hamster Anti-Mouse CD28 (Cat. No. 553294) antibodies in complete tissue culture medium. After 2 days, the cells were harvested, washed, and restimulated with Phorbol 12-Myristate 13-Acetate and Ionomycin in the presence of BD GolgiStop™ Protein Transport Inhibitor (Cat. No. 554724) for 4 hrs. The cells were then fixed and permeabilized using a BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit (Cat No. 554715) followed by staining with PerCP-Cy™5.5 Anti-Mouse CD4 (Cat. No. 550954) and FITC Anti-Mouse IL-2 (Cat. No. 554427) antibodies. Two-color flow cytometric dot plots showing the correlated expression patterns of VPD450 versus CD4 (Left Panel) or IL-2 (Right Panel) were derived from gated events with the forward and side light-scatter characteristics of intact splenic lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.)

**Application Notes**

**Application**  
**Intracellular staining (flow cytometry)** Routinely Tested

**Recommended Assay Procedure:**

**Materials Provided**  
- VPD450 dye (1 mg/vial, store desiccated at ≤ -20°C)

**Materials needed but not provided**  
- Viable single-cell suspensions of primary lymphoid cells of interest
- Fresh cell culture-grade Dimethyl Sulfoxide (DMSO; eg Sigma D2650)
- Sterile Dulbecco’s Phosphate Buffered Saline (1× PBS)
- Cell culture medium, eg with 10% Fetal Bovine Serum (FBS)
- Sterile polypropylene 15-mL (BD Falcon™ 352097) or 50-mL (BD Falcon™ 352098) conical tubes
- Microcentrifuge tubes or 2-mL cryovials
- Fluorescent antibodies for immunophenotypic or functional analysis (optional)
Required Equipment
• Violet laser-equipped BD FACSCanto™ II, BD LSRFortessa™ or BD™ LSR II Flow Cytometer
• Centrifuge
• 37°C CO2 Incubator and 37ºC Water Bath

Procedure
VPD450 has a maximum absorption of 410 nm and maximum emission of 450 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.

Preparation of VPD450 dye solution in DMSO
1. Prepare a 1 mM stock solution of VPD450 dye:
   • Add 1.0 mL of DMSO to the vial of VPD450. Mix thoroughly by vortexing.
   • Transfer the dissolved dye to a 15-mL conical tube (or any tube that will hold up to 3 mL volume).
   • Add 1.63 mL of DMSO and mix thoroughly again to ensure the dye is completely dissolved.
2. Prepare single-use aliquots of the VPD450 stock solution (user-defined volume) using appropriately labeled microcentrifuge tubes or 2-mL cryovials (the label should include the name of the dye, concentration, and date).
3. Freeze all aliquots at -80°C, protected from light.

Note: The VPD450 stock solution can be stored at -80°C for up to 6 months. Aliquots can be thawed and refrozen up to two times without significantly reducing the fluorescence intensities of VPD450-labeled cell samples.

VPD450 labeling of cells
This procedure has been optimized for labeling mouse and human lymphocytes at cell concentrations of 10-30 X 10^6/mL. If labeling fewer cells, then still follow step 6 below (ie, add 1 µL VPD450 per 1 mL of cell suspension).
1. Thaw the 1 mM stock solution of VPD450, if previously frozen.
2. Transfer cell suspensions to 15- or 50-mL polypropylene centrifuge tubes.
   Note: Avoid using polystyrene plastic containers as the dye will bind to the plastic.
3. Wash cells in 1× PBS to remove any residual serum proteins.
4. Repeat step 3.
5. Resuspend the cells thoroughly into a single cell suspension at a concentration of 10-30 X 10^6/mL in 1× PBS.
6. Add 1 µL of 1 mM VPD450 stock solution for each 1 mL of cell suspension for a final VPD450 concentration of 1 µM.
7. Incubate the dye - cell suspension in a 37ºC water bath for 10-15 minutes.
8. Add 9× the original volume of 1× PBS to the cells and pellet cells by centrifugation.
9. Decant the supernatant and gently mix to disrupt the cell pellet.
10. Add 10 mL of complete medium with 10% FBS and repeat centrifugation step.
11. Decant supernatant and gently mix to disrupt the cell pellet.
12. Resuspend the cells in complete medium and proceed to cell culture or analysis by flow cytometry.

Notes:
• Confirm brightness of the cell labeling by comparing labeled cells with unlabeled control cells.
• Immediately after labeling with VPD450, cells may exhibit fluorescence in other channels, especially the green (eg BD Horizon™ V500, AmCyan) channel, when excited by the violet laser. However, this fluorescence returns to normal levels after 24 hours.
• VPD450 can be used in intracellular staining assays that require fixation with formaldehyde and permeabilization with methanol and detergents such as those used for BD Phosflow™ staining or intracellular cytokine staining.
• Higher initial staining intensities can be achieved by increasing the initial VPD450 dye loading concentration. However, as with other succinimidyl ester containing tracking dyes, higher loading concentrations may lead to increased cell toxicity or cell death.

Product Notices
1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
3. Cy is a trademark of GE Healthcare.
4. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.

References