Technical Data Sheet

Fixable Viability Stain 450

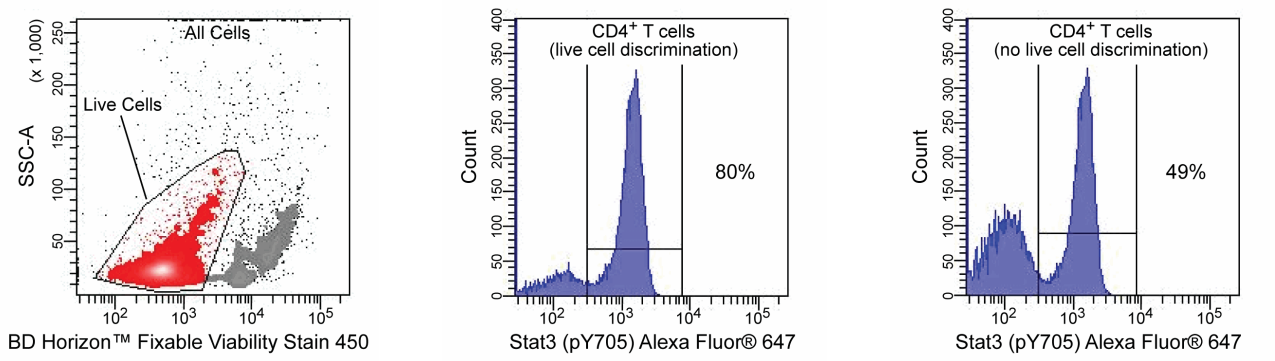
Product Information

Material Number: **562247**
Size: 0.1 mg

Description

BD Horizon™ Fixable Viability Stain 450 (FVS450) is useful to discriminate viable from non-viable mammalian cells in multicolor flow cytometric applications. This violet fluorescent stain contains a dye that reacts with and covalently binds to cell surface and intracellular amines. Permeable plasma cell membranes, such as those present in necrotic cells, allow for the intracellular diffusion of the violet dye and covalent binding to higher overall concentrations of amines than in non-permeable live cells. Therefore, necrotic cells present in a typical *in vitro* assay label with higher levels of dye increasing their fluorescence intensity 10-20 fold over that of viable cells. The labeled cells can be fixed with formaldehyde for downstream decontamination, freezing and/or permeablization and subsequent intracellular staining while maintaining stable FVS450 fluorescence.

The BD Horizon™ Fixable Viability Stain 450 is excited by the Violet laser (with an excitation maximum of 406 nm) and has a fluorescence emission maximum at 450 nm.



Multicolor flow cytometric analysis of phosphorylated STAT3 expression by "viable" activated human peripheral blood mononuclear cells (PBMC). *PBMC were cultured for 48 hours in complete tissue culture medium and then frozen and stored (-80°C) for ten days. The cells were thawed and treated with recombinant human IL-6 (100 ng/ml; Cat. No. 550071) for 15 minutes with BD Horizon™ Fixable Viability Stain 450 (Cat. No. 562247) added for the last 7 minutes of activation. Cells were then fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655) and permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050) according to the standard Phosflow protocol. Cells were stained with PE Mouse Anti-Human CD3 (Cat. No. 555333), PerCP-Cy™5.5 Mouse Anti-Human CD4 (Cat. No. 552838) and BD Phosflow™ Alexa Fluor® 647 Mouse Anti-Stat3 (pY705) (Cat. No. 557815) antibodies. The dual parameter flow cytometric dot plot (Left Panel) shows the incorporated levels of FVS450 versus side scattered light signals expressed by the PBMC. Flow cytometric histograms show the levels of Stat3 (pY705) expressed by live cell-discriminated (ie, gated events with low level FVS450 incorporation; Middle Panel) and total events (including cells with both low and high levels of FVS450; Right Panel). The CD4+CD3+ T lymphocytes were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometry was performed using a BD LSRFortessa™ Flow Cytometer System.*

FVS450 was also tested in mouse (data not shown).

Application Notes

Application

Flow cytometry	Tested During Development
Intracellular staining (flow cytometry)	Tested During Development

Recommended Assay Procedure:

Preparation

Bring FVS450 dye powder and 400 µl of fresh cell culture-grade Dimethyl Sulfoxide (DMSO; eg. Sigma D2650) to room temperature. Add 400 µl of DMSO and vortex solution well. Inspect the solution and repeat vortex until the stock dye has fully dissolved.

Storage

Upon arrival, store the dry dye at -80°C until use. After reconstitution with DMSO, store the solution at -20°C. The dye solution can be used for up to four freeze-thaw cycles. Aliquots (eg, ~100 µl aliquots) can be made and stored at -20°C when required for smaller experiments. Do not use reconstituted dye after 40 days of storage. Please discard the dye solution after 40 days post reconstitution with DMSO.

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Cytometry Requirements

Violet laser-equipped Flow Cytometers (eg, BD FACSCanto™ II, BD LSRFortessa™ or BD™ LSR II) can be used. Fluorescence compensation is best achieved using BD™ CompBeads Anti-Mouse Ig, κ/Negative Control (FBS) Compensation Particles Set (Cat. No. 552843) stained with BD Horizon™ V450 Mouse Anti-Human CD3, CD4, or CD19 antibodies. Alternatively, BD™ CompBeads Anti-Rat Ig, κ/Negative Control (FBS) Compensation Particles Set (Cat. No. 552844) stained with BD Horizon™ V450 Rat Anti-Mouse CD3, CD4, or CD19 antibodies can be used.

Procedure

Fixable Viability Stain 450 labeling of cells

1. Prepare cells for flow cytometry staining using sodium azide-free buffers.
2. Wash cells one time in sodium azide- and protein-free Dulbecco's Phosphate Buffered Saline (1X DPBS).
3. Resuspend cells at ~1-10 x 10^6 cells/ml in sodium azide- and protein-free 1X DPBS.
4. Add 1 µl of the Fixable Viability Stain 450 stock solution for each 1 ml of cell suspension and vortex immediately.
5. Incubate the mixture for 10-15 minutes at room temperature protected from light.
   Optional: Incubate the cells and dye mixtures at 2-8°C for 20-30 minutes (may be more desirable in mouse cell applications). Alternatively, incubate mixtures at 37°C for 5-7 minutes (eg, for BD Phosflow™ applications).
6. Wash cells once or twice with 2 ml of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or the equivalent.
7. Decant the supernatant and gently mix to disrupt the cell pellet.
8. Resuspend the cells in Stain Buffer (FBS) or equivalent.
9. Stain, fix and permeabilize cells as desired for downstream applications.

Notes:

- The reactivity of free dye is quenched by washing with buffer containing protein (eg, FBS or BSA) prior to staining with fluorescent antibodies.
- Fixable Viability Stain 450 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 (eg, Cat. No. 558050, BD Phosflow™ Perm Buffer III) or intracellular cytokine staining (eg, Cat. No. 554714, BD Cytofix/Cytoperm™ Fixation/Permeablization Kit).
- Cells may be stained in bulk prior to freezing or staining with fluorescent antibodies. Each user should determine the optimal concentrations of reagents and cells and conditions for the assay of interest.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 Tests	(none)
558050	Perm Buffer III	125 mL	(none)
552843	Anti-Mouse Ig, κ/Negative Control Compensation Particles Set	6 mL	187.1
552844	Anti-Rat Ig, κ/Negative Control Compensation Particles Set	6 mL	G16-510E3

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
4. BD Horizon™ V450 has a maximum absorption of 406 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.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. Cy is a trademark of Amersham Biosciences Limited.

References

Abrams B, Diwu Z, Guryev O, Aleshkov S, Hingorani R, Edinger M, Lee R, Link J, Dubrovsky T. 3-Carboxy-6-chloro-7-hydroxycoumarin: a highly fluorescent, water-soluble violet-excitable dye for cell analysis. *Anal Biochem.* 2009; 386(2):262-269. (Methodology)

Burmeister Y, Lischke T, Dahler AC, et al. ICOS controls the pool size of effector-memory and regulatory T cells. *J Immunol.* 2008; 180(2):774-782. (Methodology)

Charles ED, Green RM, Marukian S, et al. Clonal expansion of immunoglobulin M+CD27+ B cells in HCV-associated mixed cryoglobulinemia. *Blood.* 2008; 111(3):1344-1356. (Methodology)

Perfetto SP, Chattopadhyay PK, Lamoreaux L, et al. Amine reactive dyes: an effective tool to discriminate live and dead cells in polychromatic flow cytometry. *J Immunol Methods.* 2006; 313(1–2):199-208. (Methodology)

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