Technical Data Sheet

Fixable Viability Stain 575V

Product Information

Material Number: **565694**
Size: 200 µg

Description

BD Horizon Fixable Viability Stain 575V (FVS575V) is useful for discrimination of viable from non-viable mammalian cells in multicolor flow cytometric applications. This dye 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 dye and covalent binding to higher overall concentrations of amines than in nonpermeable 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 permeabilization and subsequent intracellular staining while maintaining stable viability stain fluorescence.

BD Horizon Fixable Viability Stain 575V is excited by the Violet laser (with an excitation maximum of 396 nm), and has a fluorescence emission maximum of 572 nm.

Danger:

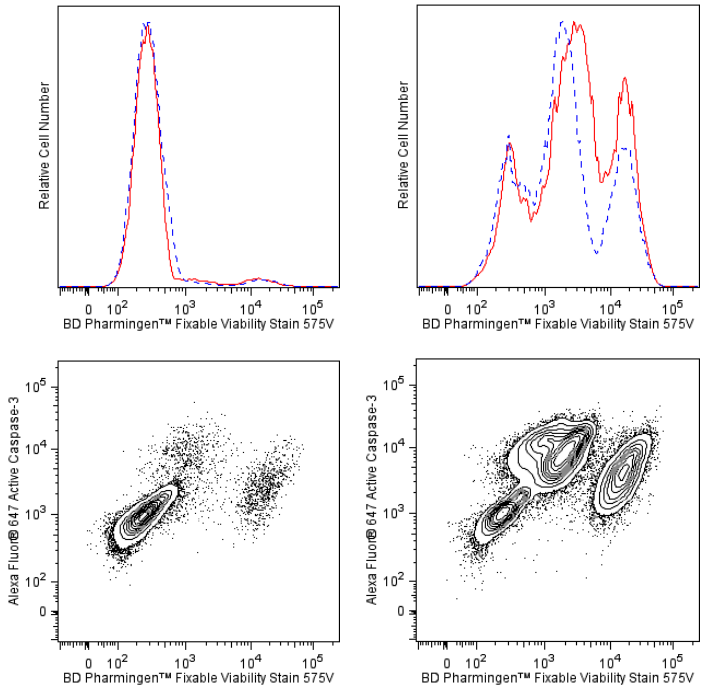
Hazard statement

Causes serious eye damage.

Precautionary statements

Wear eye protection / face protection. Wash thoroughly after handling.

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor.



Flow cytometric analysis of human Jurkat cells stained with BD Horizon™ Fixable Viability Stain 575V. *Cells from the Jurkat (Acute T cell leukemia, ATCC TIB-152) cell line were treated with 0.025% DMSO (Top Left Panel) or 5 µM camptothecin (Top Right Panel) for 16 hours and then stained with BD Horizon™ Fixable Viability Stain 575V (FVS575V; Cat. No. 565694) in serum-free buffer. The cells were then washed and either left unfixed (solid line histograms), or fixed in BD Cytofix™ Fixation Buffer (Cat. No. 554655) and permeabilized in BD Phosflow™ Perm/Wash Buffer I (Cat. No. 557885) (dashed line histograms).*

The FVS575V-stained, fixed and permeabilized cells were further stained with Alexa Fluor® 647 Rabbit Anti-Active Caspase-3 antibody (Cat. No. 560626) to identify apoptotic cells (Bottom Panels). Based on co-staining with Anti-Active Caspase-3 and FVS575V, live Jurkat cells are double negative, apoptotic Jurkat cells are Active Caspase-3-positive and FVS575-dim to mid-positive, and dead Jurkat cells are Active Caspase-3-positive and FVS575-positive.

Histograms and contour plots were derived from gated events with the forward and side light-scattering characteristics of intact Jurkat cells. Flow cytometric analysis was performed using a BD LSR II Flow Cytometry System. Please note that FVS-575V is also compatible with BD Phosflow™ Perm Buffer III (Cat. No.558050) or BD Pharmingen™ Transcription Factor Buffer Set (Cat. No. 562574/562725).

FVS575V 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 FVS575V dye powder and 340 µL of fresh cell culture-grade Dimethyl Sulfoxide (DMSO; eg, Sigma D2650) to room temperature. Add 340 µL of DMSO; vortex solution well. Inspect the solution and repeat vortex until the stock dye has fully dissolved. This is the Stock Solution.

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Storage

Upon arrival, store the dry dye desiccated and protected from light at -80°C until use. After reconstitution with DMSO, store the Stock Solution at -20°C in small aliquots. Do not use reconstituted dye after 90 days of storage. Please discard the dye solution after 90 days post reconstitution with DMSO.

Cytometry Requirements

Violet laser-equipped Flow Cytometers (eg, BD FACSCanto™ II, BD LSRFortessa™ or BD™ LSR II) can be used. Please note that FVS575V is also excited by the UV laser. This dye can be read out of filters commonly used for BD Horizon Brilliant Violet 605 (eg, 610/20) or Pacific Orange (eg, 575/20). Fluorescence compensation is best achieved using a sample of the cells of interest. When designing multicolor staining reagent panels, please be aware of spillover into the BD Horizon BV510 channel. Panels should be optimized to take this spillover into account. We recommend titrating the dye and using the lowest possible concentration that provides adequate resolution of live and dead populations for the cell type of interest to reduce spillover.

Procedure

Fixable Viability Stain 575V 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 (1× DPBS).
3. Resuspend cells at 1-10×10^6 cells/ml in sodium azide- and protein-free 1× DPBS.
4. Add 1 µL of BD Horizon Fixable Viability Stain 575V Stock Solution for each 1 ml of cell suspension (1:1000) and vortex immediately.
    a. *Note:* We recommend titrating the dye for optimal performance, as different cell types and different applications can result in a wide degree of variability in staining.
5. Incubate the mixture for 10-15 minutes at room temperature protected from light.
    a. *Optional:* Alternatively, incubate mixtures at 37°C for 5-7 minutes or 2-8°C for 30-60 minutes.
6. Wash cells 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:

1. Each user should determine the optimal concentrations of reagents, cells, and conditions for the assay of interest. We recommend titrating the reagent in early experiments to obtain optimal results.
2. The reactivity of the free dye is quenched by washing with buffer containing protein (eg, FBS or BSA).
3. Cells may be stained in bulk prior to freezing or staining with fluorescent antibodies.
4. BD Horizon Fixable Viability Stain 575V 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 (e.g., Cat. No. 558050, BD Phosflow Perm Buffer III), intracellular cytokine staining (eg, Cat. No. 554714, BD Cytofix/Cytoperm™ Fixation/Permeablization Kit), or transcription factor staining (eg, Cat. No. 562574, BD Pharmingen Transcription Factor Buffer Set).
5. Apoptotic cells can show variable staining. We recommend co-staining with, e.g., Annexin V APC (Cat. No. 550475) or Alexa Fluor® 647 Rabbit Anti-Active Caspase-3 (Cat. No. 560626) if further analysis is desired for the apoptotic cells.

Suggested Companion Products

Catalog Number	Name	Size	Clone
558050	Perm Buffer III	125 mL	(none)
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 Tests	(none)
562574	Transcription Factor Buffer Set	100 Tests	(none)
562725	Transcription Factor Buffer Set	25 Tests	(none)
554655	Fixation Buffer	100 mL	(none)
550475	APC Annexin V	200 Tests	(none)
560626	Alexa Fluor® 647 Rabbit Anti-Active Caspase-3	50 Tests	C92-605

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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

Abrams B, Diwu Z, Guryev O, et al. 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)

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Perfetto SP, Chattopadhyay PK, Lamoreaux L, et al. Amine-reactive dyes for dead cell discrimination in fixed samples. *Curr Protoc Cytom.* 2010; 9(9.34) (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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