Technical Data Sheet

Fixable Viability Stain 620

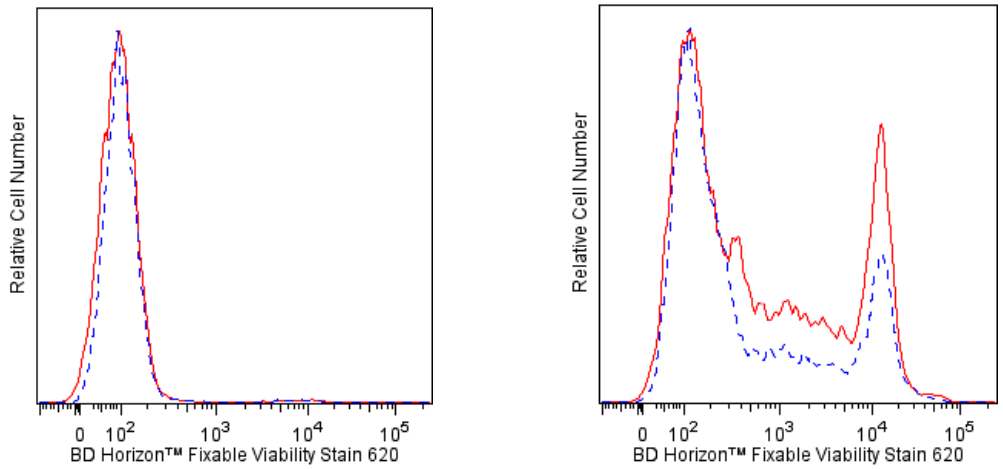
Product Information

Material Number: **564996**
Size: 100 µg

Description

BD Horizon™ Fixable Viability Stain 620 (FVS620) 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 and covalent binding of the dye to the higher levels of total cellular amines when compared to living cells with impermeable plasma membranes. Therefore, necrotic cells present in a typical *in vitro* assay label with higher levels of dye increasing their FVS620 fluorescence intensities 10-20 fold over that of viable cells. The FVS620-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 620 is best excited by the Yellow-Green laser (with an excitation maximum of 523 nm), but is also well-excited by the Blue laser. It has a fluorescence emission maximum of 617 nm.



Flow cytometric analysis of human Jurkat cells stained with BD Horizon™ Fixable Viability Stain 620. *Cells from the human Jurkat (Acute T cell leukemia, ATCC TIB-152) cell line were treated with 0.025% DMSO (Left Panel) or 5 µM camptothecin (Right Panel) for 16 hours and then stained with BD Horizon™ Fixable Viability Stain 620 (Cat. No. 564996) in serum-free buffer. The cells were then 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). Histograms were derived from gated events with the forward and side light-scattering characteristics of Jurkat cells. Flow cytometric analysis was performed using a BD LSRFortessa™ Flow Cytometry System. Please note that FVS620 is also compatible with BD Phosflow™ Perm Buffer III (Cat. No.558050) or BD Pharmingen™ Transcription Factor Buffer Set (Cat. No. 562574/562725). Fixable Viability Stain 620 has been tested on mouse (data not shown).*

Application Notes

Application

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

Recommended Assay Procedure:

Preparation

Bring FVS620 dye powder and 230 µl of fresh cell culture-grade Dimethyl Sulfoxide (DMSO; eg, Sigma D2650) to room temperature. Add 230 µl of DMSO and vortex solution well. Inspect the solution and repeat vortex until the stock dye has fully dissolved. This is the Stock Solution.

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.

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Cytometry Requirements

Yellow-Green or Blue laser-equipped Flow Cytometers (eg, BD FACSCanto™ II, BD LSRFortessa™, BD LSR™ II, or BD Accuri™ C6) can be used. This dye can be read out of filters commonly used for BD Horizon™ PE-CF594 (eg, 610/20-nm filter) or PerCP-Cy™5.5 (eg, 695/40-nm filter). Fluorescence compensation is best achieved using stained and unstained samples of the cells of interest.

Procedure

Fixable Viability Stain 620 labeling of cells

1. Prepare cells for flow flow cytometric 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 BD Horizon™ Fixable Viability Stain 620 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 620 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, BD Phosflow™ Perm Buffer III, Cat. No. 558050), intracellular cytokine staining (eg, BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit, Cat. No. 554714), or transcription factor staining (eg, BD Pharmingen™ Transcription Factor Buffer Set, Cat. No. 562574/562725).
5. Apoptotic cells can show variable staining. We recommend co-staining with other fluorescent probes such as APC Annexin V (Cat. No. 550475) if further analysis is desired for the apoptotic cells.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554655	Fixation Buffer	100 mL	(none)
557885	Perm/Wash Buffer I	125 mL	(none)
558050	Perm Buffer III	125 mL	(none)
562574	Transcription Factor Buffer Set	100 Tests	(none)
562725	Transcription Factor Buffer Set	25 Tests	(none)
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 Tests	(none)
550475	APC Annexin V	200 Tests	(none)
554657	Stain Buffer (BSA)	500 mL	(none)

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. CF™ is a trademark of Biotium, Inc.
4. Cy is a trademark of GE Healthcare.
5. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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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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)

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