BD Horizon™ Fixable Viability Stain (FVS) Reagents

Features

- Amine-reactive membrane impermeable dyes useful for live/dead discrimination
- Labeled cells can be fixed and permeabilized, making the dyes compatible with multiple downstream applications
- Dyes are retained overnight in fixed cells and can also survive cryopreservation
- Eight distinct Fixable Viability Stain (FVS) reagents allow for greater choice and flexibility in multicolor panel design

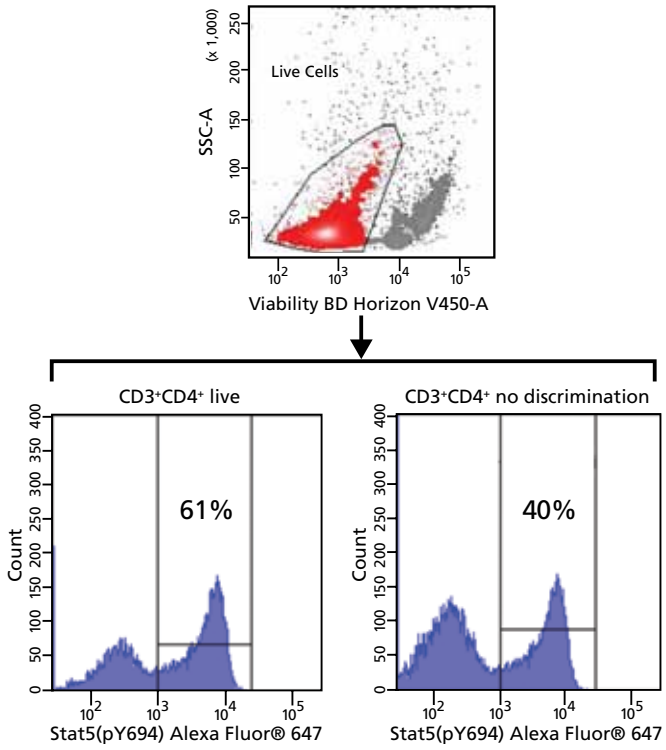

Figure 1. Multicolor flow cytometric analysis of phosphorylated Stat5 expression by "viable" activated human peripheral blood mononuclear cells (PBMCs).

PBMCs were cultured for 48 hours in complete tissue culture medium and then frozen and stored (-80°C) for 10 days. The cells were thawed and treated with recombinant human IL-2 (100 ng/mL; Cat. No. 554603) for 15 minutes with BD Horizon™ Fixable Viability Stain 450 (Cat. No. 562247) added for the last 7 minutes of activation. Cells were fixed with BD Cytofix™ fixation buffer (Cat. No. 554655), permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050), and stained with PE Mouse Anti-Human CD3 (Cat. No. 555333), PerCP-Cy™5.5 Mouse Anti-Human CD4 (Cat. No. 552838) and Alexa Fluor® 647 Mouse Anti-Stat5 (pY694) (Cat.562076). The upper plot shows the incorporated levels of FVS450 vs side scatter light signals as gated prior to using a lymphocyte gate. Intact lymphocytes, as derived from forward and side light-scatter characteristics, were further analyzed for $CD3^+CD4^+$ T lymphocyte gated events (plot not shown). The flow cytometric histograms (bottom row) show the levels of Stat5 (pY694) expressed by live cell discriminated lymphocytes vs lymphocytes for which discrimination was not applied. Flow cytometry was performed using a BD LSRFortessa™ system.

BD Biosciences continues to expand the options for multicolor flow panel design with the development of completely new BD Horizon™ Fixable Viability Stain (FVS) reagents for the violet (FVS450 and FVS510), blue (FVS520), yellow-green (FVS570 and FVS620, also excitable by the blue laser), and red lasers (FVS660, FVS700, and FVS780). BD Horizon FVS reagents are amine-reactive dyes used to discriminate viable from non-viable mammalian cells based on fluorescence intensity. The dye reacts with and covalently binds to cell surface and intracellular amines, resulting in dimly stained non-permeable live cells and more highly fluorescent cells with permeable membranes (for example, necrotic cells). Typically, dead cells have a fluorescence intensity 10 to 20-fold greater than live cells stained with the same amount of dye.

Optimized for multiple laser lines

FVS450 and FVS510 are excited by the violet laser, FVS520 by the blue laser, FVS570 and FVS620 by the yellow-green laser (also excitable by the blue laser), and FVS660, FVS700, and FVS780 by the red laser. See Table 1 for the fluorescence emission maximum for each of these reagents. These reagents are engineered for use with BD FACS™ brand flow cytometers equipped with blue, red, yellow-green, and/or violet lasers, including the BD FACSCanto™, BD FACSAria™, BD FACSVerse™, and BD™ LSR cell analyzer platforms. These reagents allow for flexibility in multicolor panel design.

Compatible with fixation and permeabilization protocols

Cells stained with FVS reagents can be fixed with a formaldehyde-based fixative and can be used in experimental protocols that require permeabilization to detect intracellular antigens. All eight FVS reagents can be used in intracellular staining assays that use alcohols or detergents for permeabilization, such as the BD Phosflow™ perm buffers, BD Cytofix/Cytoperm™ fixation/permeabilization solution, and BD Pharmingen™ transcription factor buffer. Labeled cells can also be frozen and stored for later use (Figure 2).

Tools to optimize setup, selection, and performance

Multicolor flow cytometry enables simultaneous analysis of multiple parameters in a single experiment and can quickly deliver a wealth of information about cellular composition and function. Our expanding list of reagent offerings, together with a growing library of online tools and resources from BD Biosciences, make the power of multicolor flow cytometry more accessible to researchers than ever before.

In addition to these online resources, BD Biosciences offers one-on-one technical application support as part of our comprehensive customer services. Every day around the world, BD flow cytometry experts help customers on the phone and on-site to address a range of multicolor questions from panel design to troubleshooting.

Visit **bdbiosciences.com** for more information.

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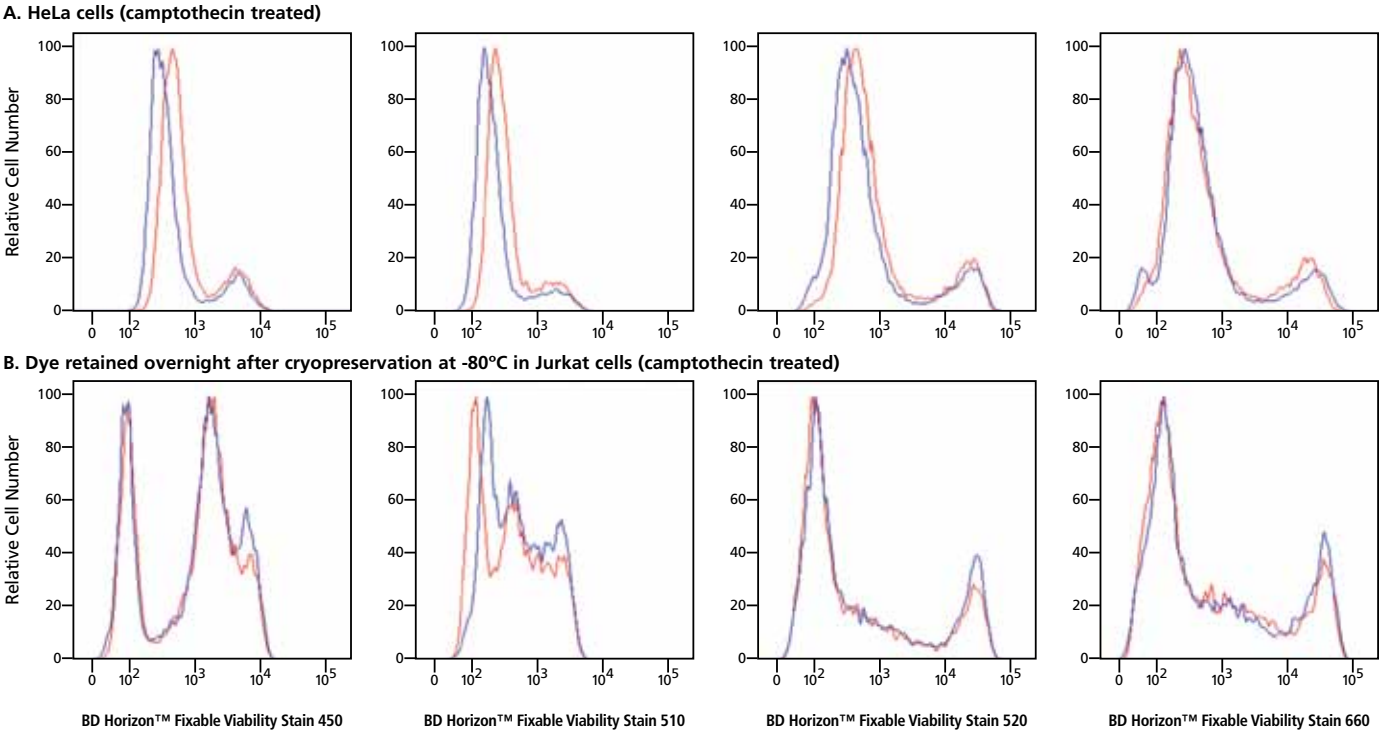

Figure 2. Fluorescent staining of BD Horizon Fixable Viability Stain reagents in HeLa or Jurkat cell lines.

HeLa (ATCC, CCL-2) **(A)** or Jurkat (ATCC, TIB-152) **(B)** cells were treated with camptothecin to induce apoptosis and death. Cell preparations were harvested and stained with BD Horizon Fixable Viability Stain reagents in serum-free buffer. Note that apoptotic cells can show an intermediate level of staining between live and dead cells. **(A)** Stained HeLa cells were unfixed (red) or fixed in BD Cytofix fixation buffer and permeabilized in BD Perm/Wash™ buffer I (Cat. No. 557885) (blue). Please note that FVS570, FVS620, FVS700, and FVS780 are also amenable to fixation and permeabilization with BD Cytofix fixation buffer and BD Perm/Wash™ buffer I.

(B) Stained Jurkat cells were fixed in fixation buffer and either acquired on a BD LSRFortessa flow cytometry system immediately (red) or cryopreserved in freezing medium for 24 hours, thawed, washed with BD Pharmingen™ stain buffer (FBS), and acquired post-cryopreservation (blue). All samples were acquired using a BD LSRFortessa flow cytometry system and histograms were derived from gated events based on light scattering characteristics of cells. Please note that FVS570, FVS620, FVS700, and FVS780 are also amenable to cryopreservation.

Ordering Information

Description	Excitation (nm)	Emission (nm)	Laser	Equivalent Fluorochromes*	Size	Cat. No.
BD Horizon™ Fixable Viability Stain 450	406	450	Violet	V450, Pacific Blue™, BV421	0.1 mg	**562247**
BD Horizon™ Fixable Viability Stain 510	408	512	Violet	V500, BV510	0.1 mg	**564406**
BD Horizon™ Fixable Viability Stain 520	498	521	Blue	FITC, Alexa Fluor® 488	0.15 mg	**564407**
BD Horizon™ Fixable Viability Stain 570	547	573	Yellow-Green/Blue	PE	0.15 mg	**564995**
BD Horizon™ Fixable Viability Stain 620	523	617	Yellow-Green/Blue	PE-CF594	0.1 mg	**564996**
BD Horizon™ Fixable Viability Stain 660	649	660	Red	APC, Alexa Fluor® 647	0.1 mg	**564405**
BD Horizon™ Fixable Viability Stain 700	657	700	Red	APC-R700, Alexa Fluor® 700	0.1 mg	**564997**
BD Horizon™ Fixable Viability Stain 780	759	780	Red	APC-Cy7	0.2 mg	**565388**

Table 1. Fixable Viability Stain reagents offered by BD Biosciences

*Do not use reagents conjugated with these fluorochromes in the same tube.

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