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
- Nine distinct Fixable Viability Stain (FVS) reagents allow for greater choice and flexibility in multicolor panel design

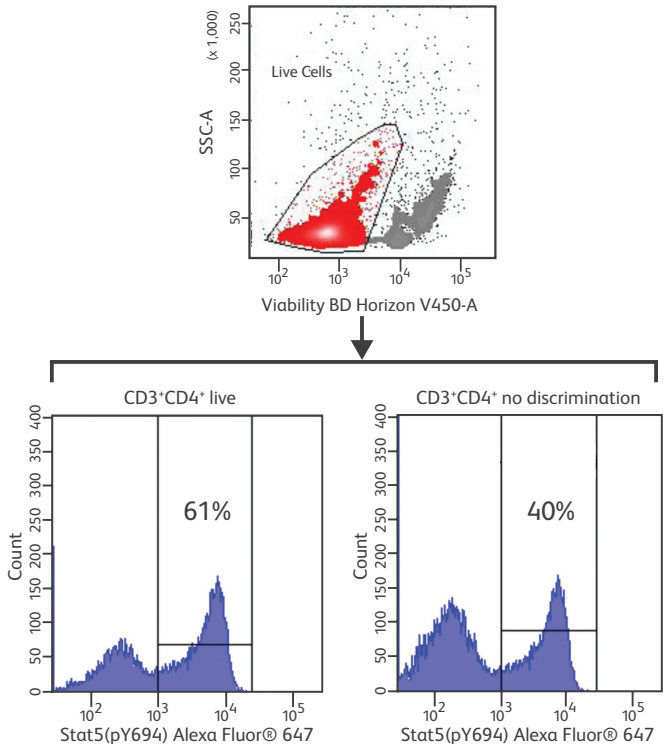


Figure 1. Multicolor flow cytometric analysis of phosphorylated Stat5 expression by activated human peripheral blood mononuclear cells (PBMCs).

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. Discrimination of dead cells allowed for a more accurate quantitation of Stat5(pY694) positive cells. Flow cytometry was performed using a BD LSRFortessa™ cell analyzer.

BD Life Sciences 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, FVS510 and FVS575V), blue (FVS520), yellow-green (FVS570 and FVS620, also excitable by the blue laser), and red lasers (FVS660, FVS700, and FVS780). See Table 1 for the fluorescence emission maximum for each of these reagents. BD Horizon FVS reagents are amine-reactive dyes used to discriminate viable from non-viable mammalian cells based on fluorescence intensity (Figure 1). 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. Apoptotic cells can vary in staining intensity, and can be identified by combining an FVS reagent with an apoptosis marker (Figure 3).

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 nine 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).

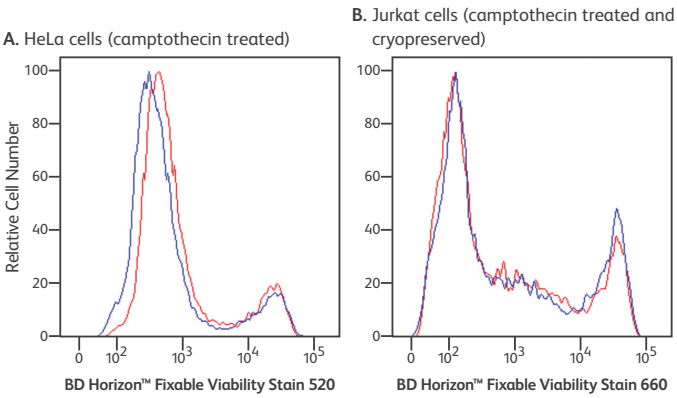


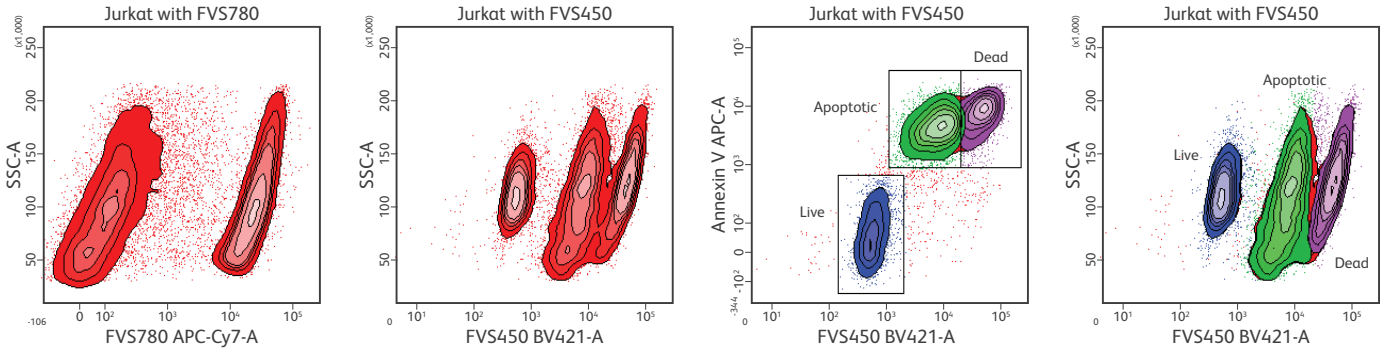
Figure 2. Fluorescent staining of BD Horizon FVS reagents in HeLa or Jurkat cell lines.

HeLa (ATCC, CCL-2) **(A)** or Jurkat (ATCC, TIB-152) **(B)** cells were treated with camptothecin to induce death and then stained with FVS in serum-free buffer. Stained HeLa cells were unfixed (red) or fixed in BD Cytofix fixation buffer and permeabilized in BD Perm/Wash™ Buffer I (blue). Stained Jurkat cells were fixed in fixation buffer and either acquired immediately (red) or cryopreserved for 24 hours at -80°C, thawed, washed, and acquired post-cryopreservation (blue). All samples were acquired using a BD LSRFortessa cell analyzer and histograms were derived from gated events based on light scattering characteristics of cells.

Visit bdbiosciences.com for more information.

BD Horizon™ Fixable Viability Stain (FVS) reagents

A. Same cell type, different BD Horizon Fixable Viability Stain



B. Different cell type, same BD Horizon Fixable Viability Stain

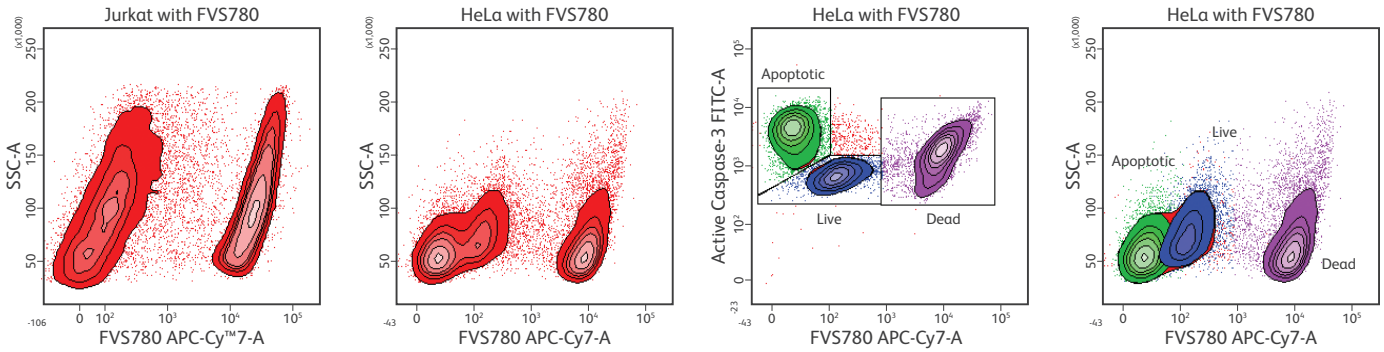


Figure 3. BD Horizon Fixable Viability Stains and apoptosis

Jurkat cells or HeLa cells were treated with 20 µM camptothecin for 16 hours and then stained with FVS780 or FVS450. Jurkat cells stained with FVS450 were further stained with BD Pharmingen™ APC Annexin V (Cat. No. 550475). HeLa cells stained with FVS780 were fixed with BD Cytofix™ Fixation Buffer, permeabilized with BD Perm/Wash™ Perm/Wash Buffer (Cat. No. 554723), and stained with BD Pharmingen™ FITC Rabbit Anti-Active Caspase-3 (Cat. No. 550480). Data were collected on a BD LSRFortessa™ cell analyzer. **(A)** Jurkat cells stained with FVS780 show two populations encompassing live/apoptotic (dim) and dead cells (bright). However, Jurkat cells stained with FVS450 show three populations: live (dim), apoptotic (intermediate), and dead (bright), as confirmed by co-staining with Annexin V. **(B)** HeLa cells stained with FVS780 also show three populations: apoptotic (dim), live (intermediate), and dead (bright), as confirmed by co-staining with Anti-Active Caspase-3. Fixable Viability Stain binding to apoptotic cells is therefore dye dependent and cell type dependent, and co-staining with an apoptosis marker such as Annexin V or Anti-Active Caspase-3 is recommended to resolve and identify apoptotic cells. This co-staining is not required if you are only interested in eliminating dead cells from your analysis.

Ordering information

Description	Excitation (nm)	Emission (nm)	Laser	Fluorescence Channel*	Size	Cat. No.
BD Horizon™ Fixable Viability Stain 440UV	338	436	UV	BUV395	0.2 mg	**566332**
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 575V	396	572	Violet	BV605, Pacific Orange™	0.2 mg	**565694**
BD Horizon™ Fixable Viability Stain 520	498	521	Blue	BB515, 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, BD™ APC-H7	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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