

BD Horizon™ Fixable Viability Stain 450 (FVS450)

Features

- Amine-reactive dye useful for live/dead discrimination
- Labeled cells can be fixed and permeabilized, making them compatible with multiple downstream applications
- Utilizes the violet laser, allowing for greater choice and flexibility in multicolor panel design

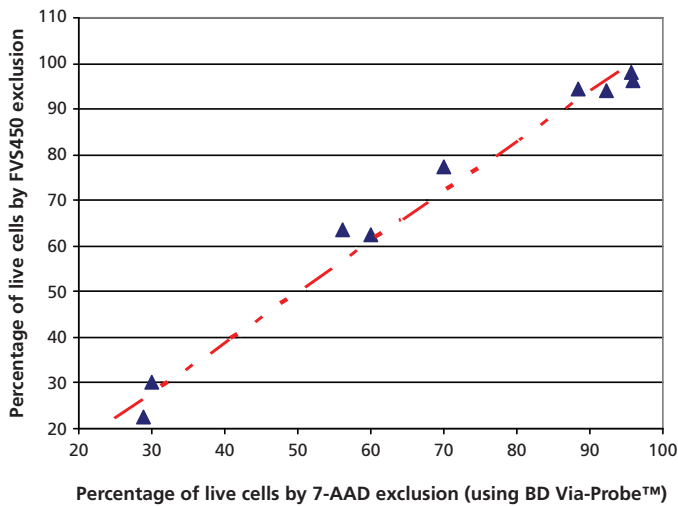


Figure 1. Correlation of viability staining results using 7-AAD versus FVS450 in various sample preparations

A linear relationship was derived from studying cell preparations and long-term in-vitro cultures. Human peripheral blood mononuclear cells (PBMCs) were treated with PMA/ionomycin (24–48 hrs) or none (resting). Mouse lymphocytes derived from spleen were treated with ConA (12 and 72 hr) or none (resting). Cell preparations were then harvested for routine flow cytometry application and stained with FVS450 and 7-AAD (BD Via-Probe) according to the respective technical data sheets (Cat. No. 562247 and 555815), then acquired by flow cytometry on either the BD FACSCanto™ II or BD LSRFortessa™ flow cytometer systems.

BD Biosciences continues to expand the options for multicolor flow panel design with the development of a completely new BD Horizon™ brand viability stain for the violet laser. The BD Horizon Fixable Viability Stain 450 (FVS450) is an amine-reactive violet dye used to discriminate viable mammalian cells from non-viable 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 (eg, 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 the violet laser

FVS450 is excited by the violet laser and has a fluorescence emission maximum at 450 nm. It is engineered for use with BD FACST™ brand flow cytometers equipped with violet lasers including the BD FACSCanto™, BD FACSAria™, BD FACSVers™, and BD™ LSR cell analyzer families. This leaves the FITC and PE channels open, maximizing choice and flexibility for multicolor panel design.

Compatible with fixation and permeabilization protocols

Once cells are stained with FVS450, they can be fixed with a formaldehyde-based fixative and used in experimental protocols that require permeabilization to detect intracellular antigens. FVS450 can be used in intracellular staining assays that use methanol or detergents for permeabilization, such as the BD Phosflow™ perm buffers or the BD Cytotfix/Cytoperm™ fixation/permeabilization solution. Labeled cells can also be frozen and stored for later use.

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/colors for more information.



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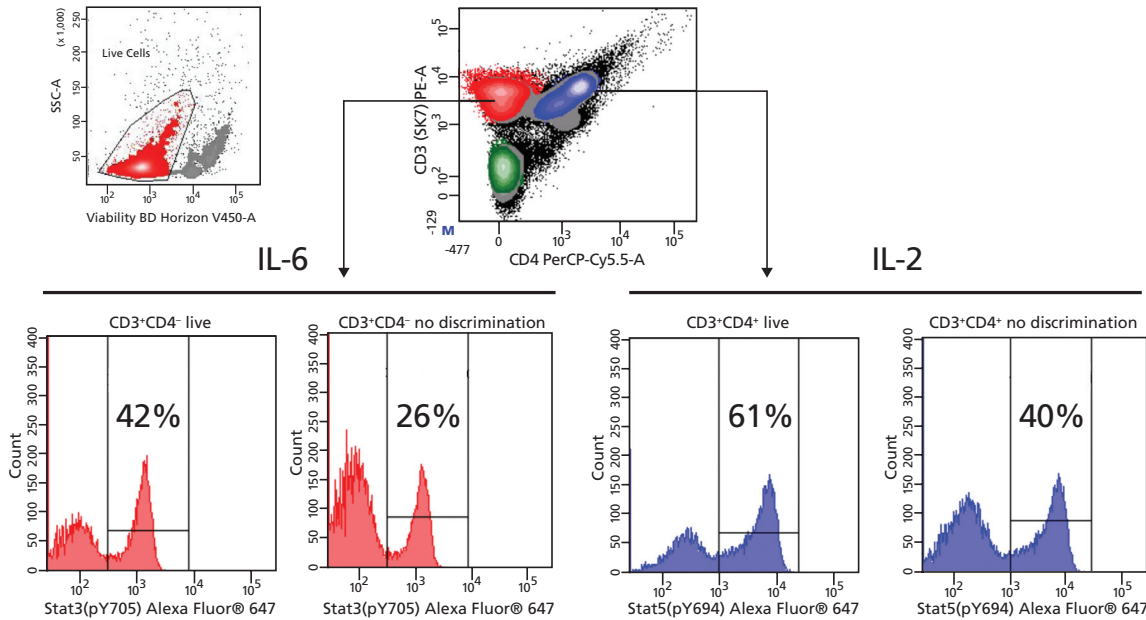


Figure 2. Examples of multicolor flow cytometric analysis of phosphorylated Stat3 and Stat5 expression by “viable” activated PBMCs.

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-6 (100 ng/mL; Cat. No. 550071) or recombinant human IL-2 (100 ng/mL; Cat. No. 554603) for 15 minutes with the FVS450 (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 BD Phosflow protocol. Two separate experiments were performed to look at phosphorylated Stat3 and Stat5. 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) or Alexa Fluor® 647 Mouse Anti-Stat5 (pY694) (Cat. No. 562076) antibodies.

The dual-parameter flow cytometric contour plot (upper left panel) shows the incorporated levels of FVS450 versus side scatter light signals as gated prior to using a lymphocyte gate. The second plot (upper right panel) shows the CD3*CD4+ and CD3*CD4+ T lymphocytes as derived from gated events with forward and side light-scatter characteristics of intact lymphocytes (gate not shown). The flow cytometric histograms (bottom row) show the levels of Stat3 (pY705) and Stat5 (pY694) expressed by live cell-discriminated lymphocytes vs lymphocytes where discrimination was not applied. Flow cytometry was performed using a BD LSRFortessa flow cytometer.

	FVS450	Propidium Iodide (PI)	7-AAD
Excitation	404 nm	351 nm	543 nm
Emission	448 nm	617 nm	647 nm
Laser(s)	405 nm	488 nm 532 nm 561 nm	488 nm 532 nm 561 nm
Mechanism	Amine-reactive	DNA intercalation	DNA/RNA intercalation
Membrane permeable	Yes	No	No
Fixable	Yes	No	No
Compatibility with intracellular staining	Yes	No	No

Table 1. Viability dyes offered by BD Biosciences.

This table compares and contrasts the properties of FVS450, PI, and 7-AAD live/dead discrimination dyes including excitation/emission, mechanism of action, and compatibility with intracellular staining.

Ordering Information

Description	Size	Cat. No.
BD Horizon Fixable Viability Stain 450	0.1 mg	562247
Propidium Iodide Staining Solution	2 mL	556463
7-AAD Staining Solution	2 mL	559925



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BD Biosciences

2350 Qume Drive
San Jose, CA 95131
US Orders: 855.236.2772
Technical Service: 877.232.8995
answers@bd.com
bdbiosciences.com