BD Horizon™ Fixable Viability Stain (FVS) Reagents
Live/Dead Cell Discrimination on the BD Accuri™ C6

Features

- Amine-reactive, membrane-impermeable dyes for live/dead cell discrimination
- Labeled cells can be fixed, permeabilized, and used in multiple downstream applications
- Dyes in fixed cells can survive overnight storage and cryopreservation
- Expand choice and flexibility in multicolor panel design

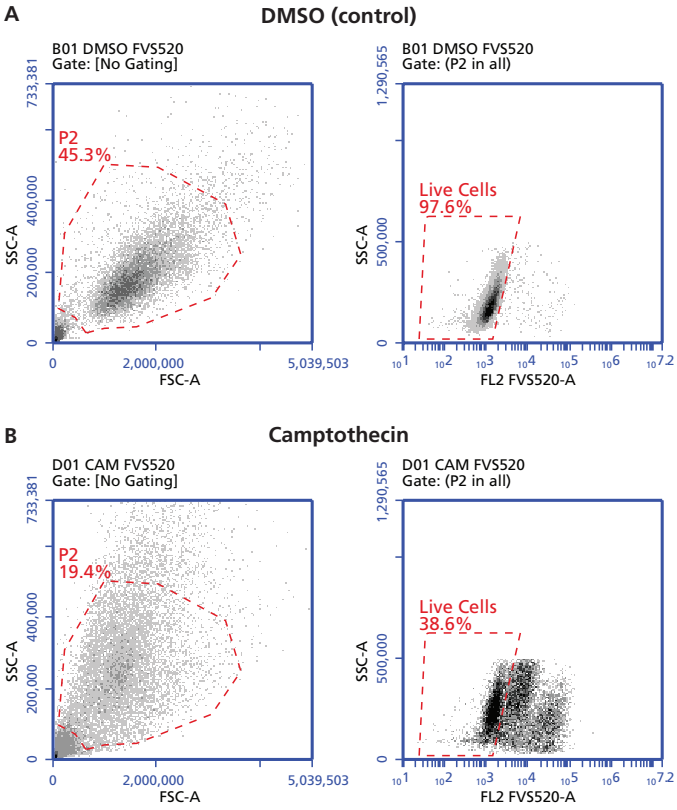

Figure 1. Distinguishing live and dead cells using BD Horizon FVS520 on the BD Accuri C6.

Jurkat cells (Human T-Cell Leukemia: ATCC TIB-152) were treated with 0.025% DMSO (control) or 5 µM of camptothecin for 20 hours to induce apoptosis. The cells were then stained with 0.1 µM (0.2X) of BD Horizon FVS520 (Cat. No. 564407) in serum-free buffer, and fixed in BD Cytofix™ fixation buffer (Cat. No. 554655). Data was acquired on a BD Accuri C6 flow cytometer and analyzed using BD Accuri™ C6 software. **Results:** Cells were initially gated based on light scatter properties (left plots). Live cells were identified using their FVS520 fluorescence profiles (right plots). As expected, camptothecin treatment **(B)** dramatically reduced the percentage of live cells compared to controls treated with DMSO **(A)**. Note that apoptotic cells can show an intermediate level of staining between live and dead cells.

BD Horizon™ Fixable Viability Stains (FVS) are amine-reactive dyes that can discriminate viable from non-viable mammalian cells based on fluorescence intensity. The dyes react with, and covalently bind to, cell surface and intracellular amines. They stain non-permeable live cells dimly, but stain cells with permeable membranes (such as necrotic cells) brightly. Typically, the fluorescence intensity of dead cells will be 10- to 20-fold higher than live cells stained with the same amount of dye.

On the BD Accuri™ C6 flow cytometer, FVS520 is excited by the blue laser and FVS660 by the red laser, allowing flexibility in multicolor panel design. Table 1 shows their fluorescence characteristics.

Unlike with traditional nucleic acid dyes such as propidium iodide (PI) and 7-amino actinomycin D (7-AAD), cells stained with FVS reagents can be fixed with a formaldehyde-based fixative and used in experimental protocols that require permeabilization to detect intracellular antigens. Both FVS reagents can be used in intracellular staining assays that use methanol 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 retain their fluorescence profiles overnight and can be frozen and stored for later use.

Easy to use, simple to maintain, and affordable, the BD Accuri C6 personal flow cytometer is equipped with a blue laser, a red laser, two light scatter detectors, and four fluorescence detectors. Compact design, fixed alignment, and pre-optimized detector settings result in a system that is simple to use, and a nonpressurized fluidics system enables kinetic measurements in real time. For walkaway convenience, the optional BD CSampler™ accessory offers automated sampling from 24-tube racks or multiwell plates.

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

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BD Horizon™ Fixable Viability Stain (FVS) Reagents

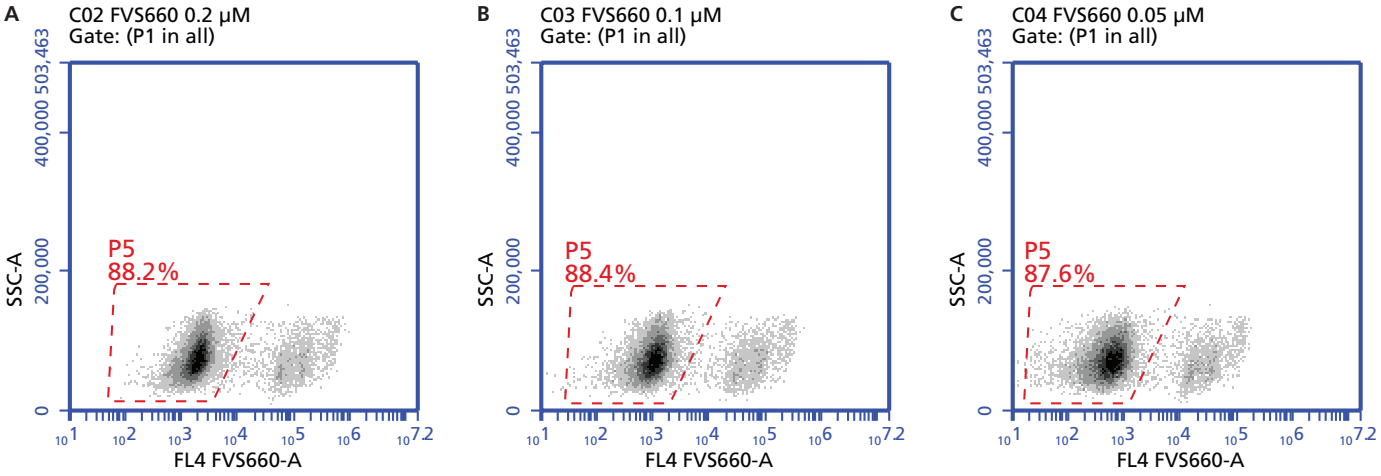

Figure 2. Distinguishing live and dead cells using varying concentrations of BD Horizon FVS660.

Human peripheral blood mononuclear cells (PBMCs) were frozen and stored at –80°C for approximately two weeks, resulting in a mixed live/dead population. Cells were thawed and stained with BD Horizon FVS660 (Cat No. 564405) at concentrations of **(A)** 0.2 µM, **(B)** 0.1 µM, and **(C)** 0.05 µM in serum-free buffer. Data was acquired on a BD Accuri C6 flow cytometer and analyzed using BD Accuri C6 software. Cells were initially gated based on light scatter properties to identify lymphocytes (not shown). **Results:** Live cells were identified using their FVS660 fluorescence profiles. Proportions of live cells were consistent across reagent concentrations, demonstrating that titration can be used to reduce background over unstained controls, which can be useful for some cell types and panel designs. Note that apoptotic cells can show an intermediate level of staining between live and dead cells. Cells can be fixed for downstream applications without loss of staining integrity.

Characteristic	FVS520	FVS660
Excitation peak	498 nm	649 nm
Emission peak	521 nm	660 nm
Laser	488 nm (blue)	640 nm (red)
Detector	FL1	FL4
Equivalent fluorochromes*	FITC Alexa Fluor® 488	APC Alexa Fluor® 647

Table 1. Fluorescence characteristics of Fixable Viability Stains on the BD Accuri C6.

**Do not use these fluorochromes in the same tube with the corresponding FVS dye.*

Ordering information

Description	Size	Cat.No.
BD Horizon™ Fixable Viability Stain 520	0.15 mg	**564407**
BD Horizon™ Fixable Viability Stain 660	0.1 mg	**564405**

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