Technical Data Sheet

# Fixable Viability Stain 520

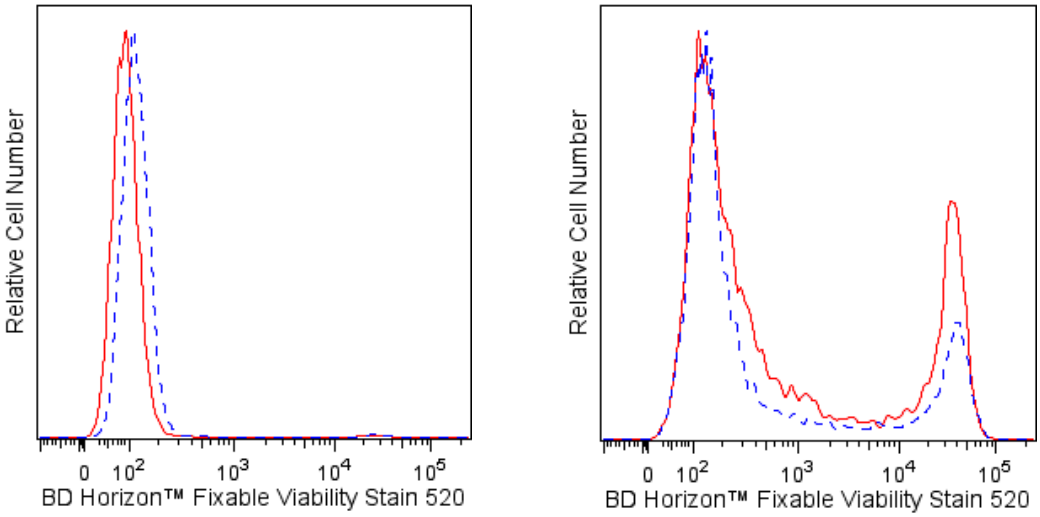
## Product Information

**Material Number:** **564407**
**Size:** 150 µg

## Description

BD Horizon™ Fixable Viability Stain 520 (FVS520) 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 of the dye and covalent binding to higher overall concentrations of amines than in non-permeable live cells. Therefore, necrotic cells present in a typical *in vitro* assay label with higher levels of dye increasing their fluorescence intensity 10-20 fold over that of viable cells. The 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 520 is excited by the Blue laser (with an excitation maximum of 498 nm) and has a fluorescence emission maximum of 521 nm.




***Flow cytometric analysis of human Jurkat cells stained with BD Horizon™ Fixable Viability Stain 520.*** *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 520 (Cat. No. 564407) 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 light scattering characteristics of intact Jurkat cells. Flow cytometric analysis was performed using a BD LSRFortessa™ Cell Analyzer System. Please note that FVS520 is also compatible with BD Phosflow™ Perm Buffer III (Cat. No. 558050) and BD Pharmingen™ Transcription Factor Buffer Set (Cat. No. 562574/562725). Fixable Viability Stain 520 has been tested on mouse (data not shown).*

## Application Notes

**Application**

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

**Recommended Assay Procedure:**

**Preparation**

Bring FVS520 dye powder and 130 µl of fresh cell culture-grade Dimethyl Sulfoxide (DMSO; e.g. Sigma D2650) to room temperature. Add 130 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**

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 FITC or Alexa Fluor® 488 (e.g. 530/30). Fluorescence compensation is best achieved using a sample of the cells of interest.

**Procedure**

**Fixable Viability Stain 520 labeling of cells**

1. Prepare cells for flow cytometry 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 1-10x10^6 cells/ml in sodium azide- and protein-free 1X DPBS.
4. Add 1 ul of BD Horizon™ Fixable Viability Stain 520 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. Please see Note 1 below for guidance on recommended ranges.
5. Incubate the mixture for 10-15 minutes at room temperature or 2-8°C protected from light.
    a. *Optional:* Alternatively, incubate mixtures at 37°C for 5-7 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. The following are ranges that we have found to work for various cell systems:
    a. Lysed Whole Blood: 1:1,000 from the Stock Solution.
    b. Primary Cells: 1:1,000 - 1:4,000 from the Stock Solution.
    c. Cell Lines: 1:4,000 - 1:10,000 from the Stock Solution.
2. The reactivity of the free dye is quenched by washing with buffer containing protein (e.g., FBS or BSA).
3. Cells may be stained in bulk prior to freezing or staining with fluorescent antibodies.
4. BD Horizon™ Fixable Viability Stain 520 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 (e.g., Cat. No. 558050, BD Phosflow™ Perm Buffer III), intracellular cytokine staining (e.g., Cat. No. 554714, BD Cytofix/Cytoperm™ Fixation/Permeablization Kit), or transcription factor staining (e.g., Cat. No. 562574, BD Pharmingen™ Transcription Factor Buffer Set).
5. Apoptotic cells can show variable staining. We recommend co-staining with, e.g., Annexin V APC (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) |
| 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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
4. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
5. 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)

Burmeister Y, Lischke T, Dahler AC, et al. ICOS controls the pool size of effector-memory and regulatory T cells. *J Immunol.* 2008; 180(2):774-782. (Methodology)

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)

Perfetto SP, Chattopadhyay PK, Lamoreaux L, et al. Amine reactive dyes: an effective tool to discriminate live and dead cells in polychromatic flow cytometry. *J Immunol Methods.* 2006; 313(1–2):199-208. (Methodology)

Perfetto SP, Chattopadhyay PK, Lamoreaux L, et al. Amine-reactive dyes for dead cell discrimination in fixed samples. *Curr Protoc Cytom.* 2010; 9(9.34) (Methodology)