Technical Data Sheet

Fixable Viability Stain 510

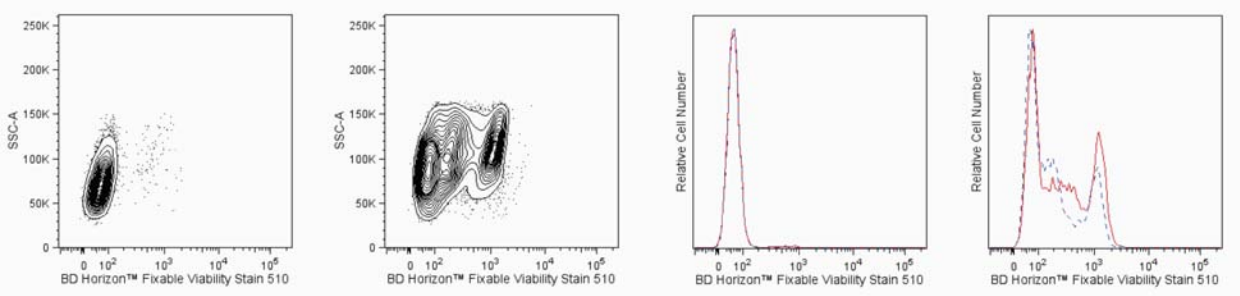
Product Information

Material Number: **564406**
Size: 100 µg
Reactivity: Tested in Development: Human, Mouse

Description

BD Horizon™ Fixable Viability Stain 510 (FVS510) 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 510 is excited by the Violet laser (with an excitation maximum of 408 nm) and has a fluorescence emission maximum of 512 nm.



Multiparameter flow cytometric analysis of human Jurkat cells stained with BD Horizon™ Fixable Viability Stain 510. *Cells from the human Jurkat (Acute T cell leukemia, ATCC TIB-152) cell line were treated with 0.025% DMSO (Left and Middle Right Panels) or 5 µM camptothecin (Middle Left and Right Panels) for 16 hours and then stained with BD Horizon™ Fixable Viability Stain 510 (Cat. No. 564406) 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 and Left Panels). Contour plots showing FVS510 fluorescence versus side-light scatter and histograms were derived from gated events based on the light scattering characteristics of intact Jurkat cells. Note that apoptotic Jurkat cells showed intermediate staining with FVS510. Flow cytometric analysis was performed using a BD LSRFortessa™ Cell Analyzer System. Please note that FVS510 is also compatible with BD Phosflow™ Perm Buffer III (Cat. No. 558050) and BD Pharmingen™ Transcription Factor Buffer Set (Cat. No. 562574/ 562725).*

Application Notes

Application

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

Recommended Assay Procedure:

Preparation

Bring FVS510 dye powder and 260 µl of fresh cell culture-grade Dimethyl Sulfoxide (DMSO; e.g. Sigma D2650) to room temperature. Add 260 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.

Cytometry Requirements

Violet laser-equipped Flow Cytometers (eg, BD FACSCanto™ II, BD LSRFortessa™ or BD™ LSR II) can be used. This dye can be read out of filters commonly used for BD Horizon™ Brilliant Violet 510, BD Horizon™ V500, or AmCyan (e.g., 525/50). Fluorescence compensation is best achieved using a sample of the cells of interest.

BD Biosciences
bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.268.5430	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Procedure

Fixable Viability Stain 510 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 1x10^6 cells/ml in sodium azide- and protein-free 1X DPBS.
4. Add 1 µl of the BD Horizon™ Fixable Viability Stain 510 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.
5. Incubate the mixture for 15 minutes at room temperature protected from light.
    a. *Optional:* Incubate the cells and dye mixtures at 2-8°C for 30-60 minutes. 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 concentations of reagents, cells, and conditions for the assay of interest. We recommend titrating the reagent in early experiments to obtain optimal results.
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 510 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)
557885	Perm/Wash Buffer I	125 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)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

References

Barny Abrams, Zhenjun Diwu, Oleg Guryev, Sergei Aleshkov, Ravi Hingorani, Mark Edinger, Rita Lee, Joe Link, Tim Dubrovsky. 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)

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.268.5430	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**