Technical Data Sheet

Yellow-Green Live Cell Caspase Probe

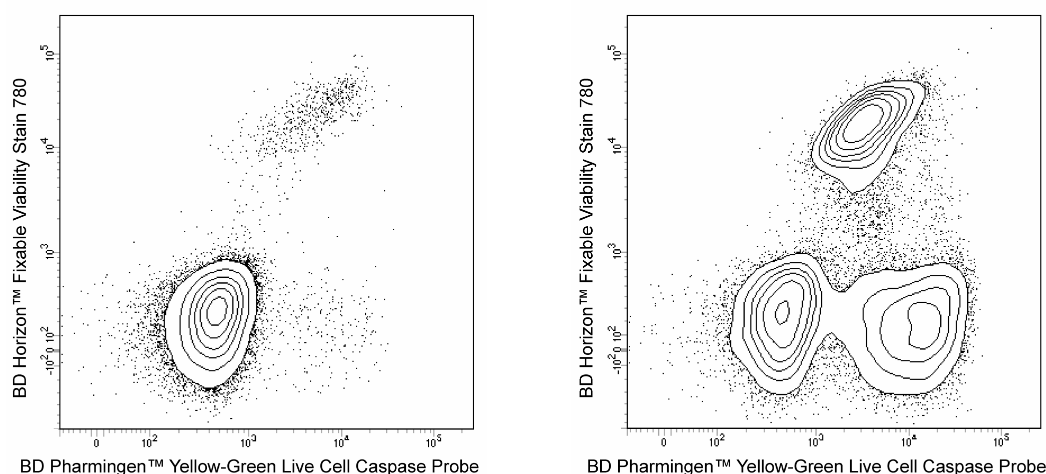
Product Information

Material Number: **565520**
Size: 50 Tests

Description

Caspases are critical mediators of apoptosis, and also play roles in other forms of cell death and inflammation. In healthy cells, caspases exist in inactive, pro-enzyme forms. During apoptosis, caspases become activated by cleavage and then proceed to initiate apoptosis. Cells with active caspases can be detected by the BD Pharmingen™ Yellow-Green Live Cell Caspase Probe, which contains a fluorochrome-labeled caspase inhibitor. Fluorochrome-labeled caspase inhibitors have three functional domains: (1) a fluorochrome , (2) a 3-amino acid sequence (VAD) that binds to the active site of the activated caspase, and (3) a fluoromethyl ketone (FMK) moiety that allows the probe to irreversibly bind the active caspase. The VAD moiety allows binding of these probes to most of the caspase family (caspase-1, -2, -3, -6, -8, -9, and -10), allowing detection of general caspase activity.

BD Pharmingen™ Yellow-Green Live Cell Caspase Probe is excited by the yellow-green laser (with an excitation maximum of 556 nm), and has a fluorescence emission maximum of 575 nm. The probe is also well-excited by the blue laser.



Two-color flow cytometric analysis of Viability and Caspase Activity in Camptothecin-treated Jurkat Cells. *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. The cultured cells were then stained with BD Pharmingen™ Yellow-Green Live Cell Caspase Probe (Cat. No. 565520) according to the recommended assay procedure. After recovery in media, cells were resuspended in DPBS and stained with BD Horizon™ Fixable Viability Stain 780 (FVS780 , Cat. No. 565388). Two-color flow cytometric contour plots showing the correlated expression of Yellow-Green Live Caspase Probe fluorescence versus BD Horizon™ Fixable Viability Stain 780 fluorescence were derived from gated events with the forward and side light-scatter characteristics of viable cells. Live cells are double negative for both fluorescent probes, whereas apoptotic cells are positive for Yellow-Green Live Cell Caspase Probe and negative for FVS780, and dead cells are positive for FVS780. Note that dead cells bind intermediate amounts of Yellow-Green Live Cell Caspase Probe. Flow cytometric analysis was performed using a BD™ LSRFortessa™ Cell Analyzer System. Yellow-Green Live Cell Caspase Probe has been tested on mouse (data not shown).*

Preparation and Storage

Store at -20°C, protected from exposure to light.

Application Notes

Application

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

Recommended Assay Procedure:

Note: Before staining, confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.

Preparation

Bring lyophilized probe and 50 µl of fresh cell culture-grade Dimethyl Sulfoxide (DMSO; e.g. Sigma D2650) to room temperature. Add 50 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.

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Storage

Upon arrival, store the dry probe desiccated and protected from light at -20°C until use. After reconstitution with DMSO, store the Stock Solution at -20°C in small aliquots. The Stock Solution is stable for at least 3 months post reconstitution with DMSO.

Cytometry Requirements

Blue or yellow-green laser-equipped Flow Cytometers (eg, BD FACSCanto™ II, BD LSRFortessa™, BD™ LSR II, and BD Accuri™ C6) can be used. This dye can be read out of filters commonly used for PE (eg, 575/26 nm) or BD Horizon™ PE-CF594 (eg, 610/20 nm). Fluorescence compensation is best achieved using a sample of the cells of interest stained with the dye. When designing multicolor panels, we recommend titrating the dye and using the lowest possible concentration that provides adequate resolution of positive and negative populations for the cell type of interest to reduce spillover.

Procedure

Labeling of Cells with BD Pharmingen™ Yellow-Green Live Cell Caspase Probe

1. Count cells to determine cell density. Adjust cell density to 1-2 × 10^6 cells/ml in fresh, pre-warmed cell culture media.
    a. Adherent cells may be stained either *in situ* or in suspension after removal from the growth substrate. Cells should be 70% confluent or less when assayed.
    b. Note: One “test” is defined as a 500 µL aliquot of cells at 1-2×10^6 cells/ml.
2. Add 2 µL of Stock Solution per 1 mL of cell suspension or culture media. For example, for a 500 µL aliquot of cells, add 1 µL of the Stock Solution. Mix thoroughly.
    a. In some cases, it may be useful to titrate the probe, as different cell types and different applications can result in variability in staining intensity.
3. Incubate samples for 30-60 minutes at 37°C protected from light.
4. Wash cells once with fresh, pre-warmed media.
5. Resuspend cells in fresh, pre-warmed media and incubate cells for an additional 15-60 minutes at 37°C protected from light to allow unbound probe to diffuse out of the cells.
6. Pellet cells once more by centrifugation and remove the supernatant.
7. Resuspend the cells in 1× Dulbecco’s Phosphate Buffered Saline (DPBS) or equivalent.
    a. Note: Dead cells bind variable amounts of probe. We recommend co-staining with, eg, BD Via-Probe™ Cell Viability Solution (Cat. No. 555816) in order to distinguish dead cells from live or apoptotic cells.
8. Analyze samples by flow cytometry.

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.
2. BD Pharmingen™ Yellow-Green Live Cell Caspase Probe is compatible with fixation and permeabilization protocols such as those used for BD Phosflow™ staining (eg, Cat. No. 558050, BD Phosflow™ Perm Buffer III) or intracellular cytokine staining (eg, Cat. No. 554714, BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit). However, please note that resolution of positive and negative populations is reduced in fixed and permeabilized cells as compared to live cells. Where possible, it is recommended that samples be analyzed live.

Suggested Companion Products

Catalog Number	Name	Size	Clone
555815	Cell Viability Solution	500 Tests	(none)
555816	Cell Viability Solution	100 Tests	(none)
565388	Fixable Viability Stain 780	200 µg	(none)
564907	DAPI Solution	1 mg	(none)
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 Tests	(none)
558050	Perm Buffer III	125 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. CF™ is a trademark of Biotium, Inc.
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

Amstad PA, Yu G, Johnson GL, Lee BW, Dhawan S, Phelps DJ. Detection of caspase activation in situ by fluorochrome-labeled caspase inhibitors. *Biotechniques.* 2001; 31(3):608. (Methodology)

Bedner E, Smolewski P, Amstad P, Darzynkiewicz Z. Activation of caspases measured in situ by binding of fluorochrome-labeled inhibitors of caspases (FLICA): correlation with DNA fragmentation. *Exp Cell Res.* 2000; 259(1):308-313. (Methodology)

Grabarek J, Amstad P, Darzynkiewicz Z. Use of fluorescently labeled caspase inhibitors as affinity labels to detect activated caspases. *Hum Cell.* 2002; 15(1):1-12. (Methodology)

Wlodkowic D, Telford W, Skommer J, Darzynkiewicz Z. Apoptosis and beyond: cytometry in studies of programmed cell death. *Methods Cell Biol.* 2011; 103:55-98. (Methodology)