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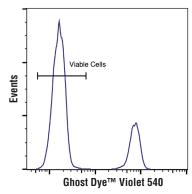
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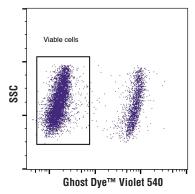
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Applications

Species Cross-Reactivity AII

Description: Ghost Dye™ Violet 540 Fixable Viability Dye is used to discriminate viable from non-viable mammalian cells in flow cytometry applications. Ghost Dye™ Violet 540 Fixable Viability Dye irreversibly binds free amines available on the cell surface as well as intracellular free amines exposed in cells with compromised cell membranes. Non-viable cells with loss of membrane integrity will be labeled with significantly more dye than healthy cells in the same sample. The cells may then be fixed, and the degree of labeling will be preserved through fixation, permeabilization, and antibody incubation steps. Cells that exhibit increased fluorescence intensity were non-viable at the time of fixation and can be excluded from analysis.





Flow cytometric analysis of live and fixed/permeabilized peripheral blood mononuclear cells, combined and stained with Ghost Dye™ Violet 540 Fixable Viability Dye. Viable gate is indicated.

Storage: Store at -20°C dessicated and protected from light. This product is stable for 12 months. Aliquot to avoid excessive freeze-thaw cycles.

Directions For Use:

- 1. Prepare the following reagents with reverse osmosis deionized (RODI) or equivalent grade water:
 - a. 1X PBS (azide- and protein/serum-free)
 - b. Incubation Buffer: Dissolve 0.5 g Bovine Serum Albumin (BSA) (#9998) in 100 ml 1X PBS. Store at 4°C.
- 2. Remove Ghost Dye™ from -20°C and bring to room temperature.
- **3.** Collect cells by centrifugation and aspirate supernatant.
- 4. Wash cells by centrifugation in excess 1X PBS. Repeat if
- **5.** Resuspend cells to a concentration of 1-10 x 10⁶/mL in 1X
- 6. Centrifuge the Ghost Dye™ before opening then add 1 uL for each 1 mL of cell suspension and vortex immediately.
- 7. Incubate for 30 minutes at 4°C protected from light.
- 8. Wash by centrifugation in excess incubation buffer. Discard supernatant. Repeat.
- 9. Cells can then be fixed, permeabilized, and immunostained based upon experimental design and recommended
- **10.** Exclude cells with high Ghost Dye™ fluorescence from analysis. These were non-viable cells at the time of fixation. See details below for excitation and emission specifications.

Ghost Dye™ Violet 540 Fixable Viability Dye excited by the violet (405 nm) laser line and has a peak emission of 540 nm that can be detected using a 525/50 band pass filter commonly used for detection of AmCyan.

Ghost Dye is a registered trademark of Tonbo Biosciences.

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