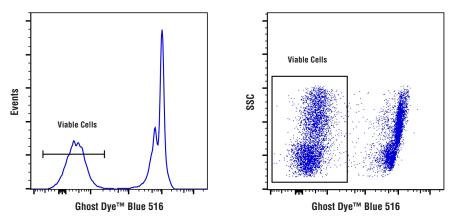




Applications	Species Cross-Reactivity
F	AII

Description: Ghost Dye™ Blue 516 Fixable Viability Dye is used to discriminate viable from non-viable mammalian cells in flow cytometry applications. Ghost Dye™ Blue 516 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 heat killed mouse bone marrow cells, combined and stained with Ghost Dye™ Blue 516 Fixable Viability Dye. Viable gate is indicated. **Storage:** Store at -20°C desiccated 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.
- Remove Ghost Dye[™] from -20°C and bring to room temperature.
- 3. Collect cells by centrifugation and aspirate supernatant.
- Wash cells by centrifugation in excess 1X PBS. Repeat if necessary.
- 5. Resuspend cells to a concentration of 1-10 x 10⁶/mL in 1X PBS.
- 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.
- Cells can then be fixed, permeabilized, and immunostained based upon experimental design and recommended protocols.
- 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™ Blue 516 Fixable Viability Dye excited by the Blue (488 nm) laser line and has a peak emission of 516 nm that can be detected using the recommended 530/30 band pass filter commonly used for detection of FITC.

Ghost Dye is a registered trademark of Tonbo Biosciences.

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse AII—all species expected Species enclosed in parentheses are predicted to react based on 100% homology