

# Product Information

## Live-or-Dye™ Fixable Viability Staining Kits

### Kit Contents

Component	50 labeling reactions (Trial size)	200 labeling reactions
Fixable Dead Cell Stain	Component A 1 vial	Component A 4 vials
Anhydrous DMSO	99953 150 uL	99953-1 250 uL

**Spectral Properties:** See Table 1.

### Storage and Handling

Store the solid dye and anhydrous DMSO at -20°C, desiccated and protected from light. When stored as directed, solid dye is stable for at least 6 months from the date of receipt. Once reconstituted in anhydrous DMSO, leftover dye can be stored at -20 °C for at least one month.

### Product Description

Live-or-Dye™ Fixable Viability Staining Kits are designed for discrimination between live and dead cells during flow cytometry or microscopy. Live/dead stains are useful probes to include when analyzing cell surface protein expression by flow cytometry, because they allow intracellular fluorescence signal from dead cells with permeable plasma membranes to be excluded from analysis.

Live-or-Dye™ Fixable Viability Stains are cell membrane impermeable amine-reactive dyes. The dyes are able to enter into dead cells that have compromised membrane integrity and covalently label free amines on intracellular proteins. On live cells, the dyes react with surface proteins, but these are much less abundant than intracellular proteins, resulting in low staining levels compared to dead cells (Figures 1, 2 and 3). The dye labeling is extremely stable, allowing the cells to be fixed and permeabilized without loss of fluorescence or dye transfer between cells. The Live-or-Dye™ staining protocol has been optimized to maximize live/dead discrimination with minimal live cell staining (Figure 2), in order to prevent interference with immunostaining. Biotium offers a selection of eight different Live-or-Dye™ viability stains spanning the fluorescence spectrum (Table 1 and Figure 2), for maximal flexibility in multi-color analysis.

**Table 1. Spectral properties of Live-or-Dye™ Fixable Viability Stains.**

Catalog No.	Product Description	Laser line	Emission filter	Abs/Em maxima
32002, 32002-T	Live-or-Dye™ 350/448	355 nm	DAPI or Violet	347/448 nm
32003, 32003-T	Live-or-Dye™ 405/452	405 nm	Pacific Blue	408/452 nm
32009, 32009-T	Live-or-Dye™ 405/545	405 nm	AmCyan	395/545 nm
32004, 32004-T	Live-or-Dye™ 488/515	488 nm	FITC	490/515 nm
32005, 32005-T	Live-or-Dye™ 568/583	488 or 561 nm	PE	562/583 nm
32006, 32006-T	Live-or-Dye™ 594/614	488 or 561 nm	PE-TexasRed	561/624 nm
32007, 32007-T	Live-or-Dye™ 640/662	633 or 640 nm	APC	642/662 nm
32008, 32008-T	Live-or-Dye™ 750/777	633 or 640 nm	APC-Cy7	755/777 nm

## Experimental Protocols

### Dye Reconstitution

Remove one vial of dye and the anhydrous DMSO from the freezer and bring to room temperature. Add 50 uL of anhydrous DMSO to the vial, vortexing or pipetting up and down to ensure that all of the dye has dissolved. Once dissolved, the dye should be used within a few hours. Leftover dye solution can be aliquoted and stored desiccated at -20°C for at least 1 month.

### Cell Staining for Live/Dead Discrimination by Flow Cytometry

This staining protocol was optimized using the human Jurkat lymphocyte cell line. The protocol may need to be optimized for other cell types.

- Grow cells in culture as required for your experiment. For adherent cells, detach from the plate using trypsin or a cell dissociation reagent. Count the cells. It is desirable to use at least  $1 \times 10^6$  cells per staining reaction.
- Optional: if positive control (dead) cells are needed, incubate cells at 56°C for 45 minutes, then allow to cool to room temperature and proceed with the protocol.
- Pellet the desired number of cells by centrifugation at 350 xg for five minutes and gently pour off supernatant. For all subsequent steps, pellet cells by centrifugation after each incubation or wash.
- Wash cells once in PBS, and resuspend in PBS at a concentration of  $1 \times 10^6$  cells/mL.
 

**Note:** Do not wash or resuspend cells in FACS wash buffer containing BSA or serum at this step, because the protein in the FACS wash buffer could interfere with subsequent Live-or-Dye™ staining.
- Aliquot cells into FACS tubes, 1 mL ( $1 \times 10^6$  cells) per tube.
- Add 1 uL of Fixable Dead Cell Stain to 1 mL cells and immediately mix well.
- Incubate for 30 minutes at room temperature or on ice, protected from light.
- Wash cells once with 1 mL PBS.
 

**Note:** To stain for surface antigens, proceed to step 9. For fixation and intracellular staining, skip to step 10. Otherwise, skip to step 13.
- Stain for surface antigens:
  - Add the appropriate primary antibodies to cells in PBS.
  - Incubate for 15 minutes on ice or at room temperature in the dark.
  - Wash cells twice with 1 mL PBS.
  - If necessary, repeat steps a-c with the appropriate secondary antibodies.
  - Proceed to step 10 for fixation, otherwise, skip to step 13.

- Fix cells in 2-4% formaldehyde for 20 minutes at room temperature, or follow the recommended fixation protocol of your preferred flow cytometry fixation/permeabilization kit (see related products).
 

**Note:** For intracellular staining, other fixation methods may be optimal for specific antibodies. Because Live-or-Dye™ staining is covalent, it is compatible with commonly used fixation methods.

- Wash cells twice with 1 mL FACS wash buffer (PBS with 1% bovine serum albumin, or similar buffer). Proceed to step 12 for intracellular staining, otherwise, skip to step 13.

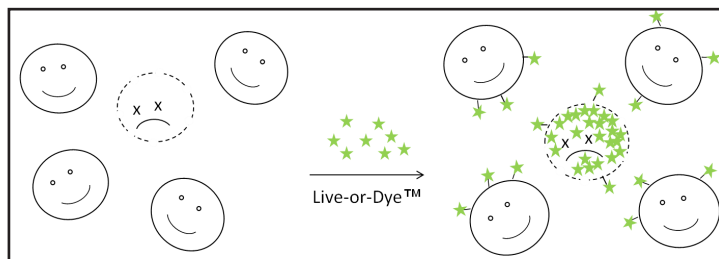


Figure 1. Principle of live/dead cell discrimination using Live-or-Dye™ Fixable Viability Stains. Live-or-Dye™ Fixable Viability Stains are cell-membrane impermeable amine-reactive dyes. The dyes can penetrate the compromised cell membranes of dead cells and label intracellular proteins. In live cells, the dyes only label cell surface proteins, resulting in much lower fluorescence signal compared to dead cells. The dye labeling is covalent, allowing cells to be fixed and permeabilized for subsequent intracellular antibody staining without loss of fluorescence or dye transfer between cells.

12. Perform intracellular staining:
  - a. Resuspend cells in 100  $\mu$ L PBS + 0.1% Triton X-100 or your preferred permeabilization buffer.
  - b. Add the appropriate primary antibodies to cells in permeabilization buffer.
  - c. Incubate for 30 minutes at room temperature in the dark.
  - d. Wash twice with 1 mL FACS wash buffer (see step 11).
  - e. If necessary, add the appropriate secondary antibodies to cells in wash buffer and repeat steps c-d.
13. Resuspend cells in 1 mL PBS or FACS wash buffer (see step 11) and analyze by flow cytometry in the appropriate channels (see Table 1).
 

**Note:** Stained and fixed cells may be stored at 4°C in the dark for several days prior to analysis.

### Quick Protocol for Live/Dead Discrimination by Microscopy

This staining protocol was optimized using the adherent human HeLa cell line. The protocol may need to be optimized for other cell types. So far four of the dyes have been validated for use in microscopy: Live-or-Dye™ 488/515, Live-or-Dye™ 568/583, Live-or-Dye™ 594/614, and Live-or-Dye™ 640/662.

1. Grow cells in culture as required for your experiment. For adherent cells, staining can be done in a chamber slide, in a multiwell plate, or on a cover slip.
2. Optional: If a positive control well containing a mixture of live and dead cells is desired, to that well add ethanol to a final concentration of 15%, incubate for 10 minutes, and wash once with PBS. Replace with PBS or growth media and proceed with the protocol.
3. Wash cells with PBS and replace media with PBS containing 1X Fixable Dead Cell Stain. Alternatively, the dye can be added directly to the culture medium. We recommend first diluting the dye stock solution in a small volume of medium before adding to cells to avoid exposing cells to a transient localized high dye concentration. For example, immediately before use, add 1  $\mu$ L dye to 100  $\mu$ L medium, then add the entire volume to cells in 1 mL culture medium
4. Incubate cells for 30 minutes at room temperature or on ice, protected from light.
5. Wash cells once with PBS.
 

**Note:** To fix and permeabilize cells for immunofluorescence, proceed to step 6. For live cell imaging, skip to step 11.
6. Fix cells in 4% paraformaldehyde for 15 minutes at room temperature, protected from light.
7. Wash cells twice with PBS.
8. Permeabilize with 0.1-0.5% Triton X-100, 5-10 min.
9. Proceed with the immunostaining of your choice. Cells can also be stained with an appropriate DNA dye such as DAPI (40043), Hoechst (40046), or RedDot2 (40061).
10. Wash cells once more in PBS.
11. Cells can be imaged immediately on the chamber slide or dish, or alternatively can be mounted using an antifade mounting medium such as EverBrite Mounting medium (23002) if desired.

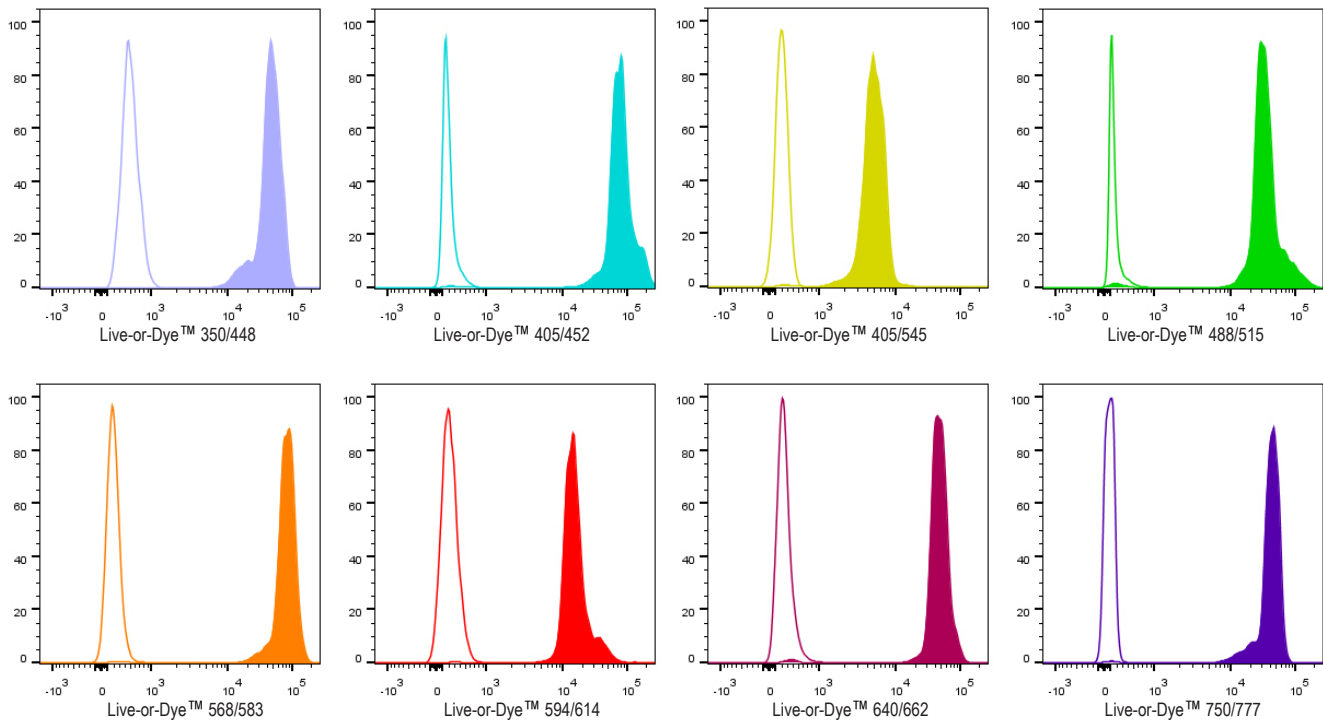


Figure 2. Discrimination of live and dead cells by flow cytometry using Live-or-Dye™ Fixable Viability Stains. Jurkat cells were either left untreated or killed by heating to 56°C for 45 minutes, then stained according to the product protocol with the Live-or-Dye™ cell stain shown on each histogram x-axis. Fluorescence was analyzed on a BD LSRII flow cytometer (see Table 1 for recommended laser/filter combinations). Heat killed cells (solid peaks) showed much higher fluorescence intensity compared to live cells (white peaks), allowing the two populations to be clearly distinguished. Results are shown for unfixed cells; nearly identical histograms were observed after cell fixation with 2% formaldehyde in PBS for 20 minutes at room temperature, followed by permeabilization with 0.1% Triton X-100 in PBS for 30 minutes at room temperature. Fluorescence histograms for live cells and dead cells were overlaid using FlowJo data analysis software.

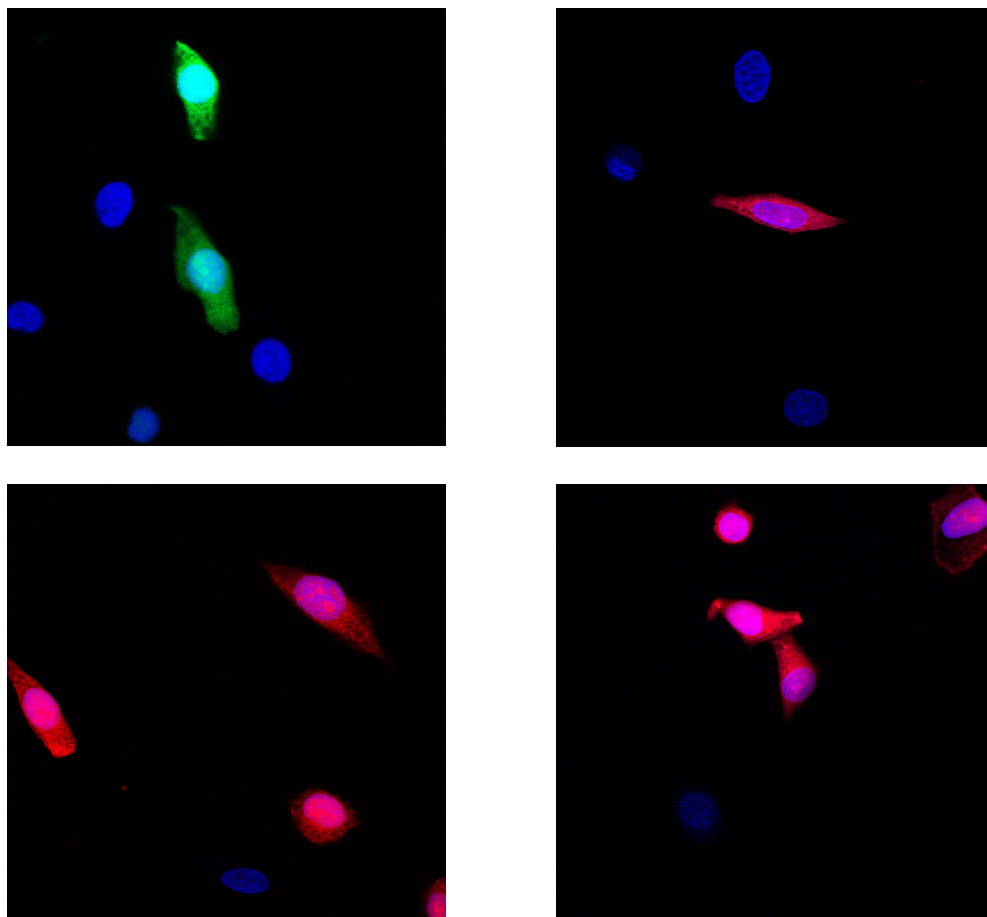


Figure 3. Discrimination of live and dead cells in fluorescence microscopy using Live-or-Dye™ Fixable Viability Stains. HeLa cells were seeded on chambered cover glasses; 18-24 hours after plating they were treated with 15% ethanol for 10 minutes to kill a subset of the cells. The cells were stained according to the product protocol with one of four Live-or-Dye™ cell stains: Live-or-Dye™ 488/515 (upper left), Live-or-Dye™ 568/583 (upper right), Live-or-Dye™ 594/614(lower left), or Live-or-Dye™ 640/662 (lower right). All cells were then stained with Hoechst to label both live and dead cells. Cells were imaged on a Zeiss LSM 700 confocal microscope. Ethanol-killed cells show Live-or-Dye™ fluorescence staining compared to live cells (blue Hoechst-stained nuclei), allowing the two populations to be clearly distinguished. Results are shown for cells fixed with 4% formaldehyde in PBS for 20 minutes at room temperature, followed by permeabilization with 0.1% Triton X-100 in PBS for 30 minutes at room temperature.

#### Related Products

Cat.#	Product Name	Unit Size
30069	AccuEasy™ Flow Cytometry Kit	1 kit
22003	Mini Cell Scrapers	200 items
30068	ViaFluor™ 405-SE Cell Proliferation Assay Kit	1 kit
23006	Flow Cytometry Fixation/Permeabilization Kit	50 tests
22015	Fixation Buffer	100 mL
22016	Permeabilization Buffer	100 mL
22017	Permeabilization and Blocking Buffer	100 mL
40048	Propidium Iodide Buffer (50 ug/mL)	2 mL
30029	NucView™ 488 Caspase-3 Assay Kit for Live Cells	100 assays
30065	Apoptosis and Necrosis Quantitation Kit Plus	50 assays
40060	RedDot1 Far-Red Nuclear Stain, 200X in H2O	150 assays
40061	RedDot2 Far-Red Nuclear Stain, 200X in DMSO	150 assays
40046	Hoechst 33342, 10 mg/mL in H2O	10 mL
23002	EverBrite Mounting Medium with DAPI	10 mL
23004	EverBrite Hardset Mounting Medium with DAPI	10 mL

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