Revised: February 29, 2016

Product Information

Live-or-Dye NucFix™ Red Staining Kit

Catalog Number: 32010, 32010-T

Unit Size: 200 assays, 50 assays (trial size)

Kit Contents

Component	50 labeling reacions (trial size)	200 labeling reactions
Live-or-Dye NucFix Red Dye	32010A 1 vial	32010A 4 vials
Anhydrous DMSO	99953 150 uL	99953-1 250 uL

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 1 year from the date of receipt. Once reconstituted in anhydrous DMSO, leftover dye can be stored dessicated at -20 °C for at least one month.

Spectral Properties

Ex/Em maxima: 520/610

Product Description

Biotium's line of 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. In microscopy, live/dead stains allow unambiguous visual discrimination of dead cells.

Live-or-Dye NucFix™ Red is a unique, cell membrane impermeable dye that specifically stains the nuclei of dead cells (Figure 1). The dye is able to enter into dead cells that have compromised membrane integrity and covalently label the cell nucleus, allowing for clear differentiation of live and dead cells by either microscopy or flow cytometry (Figures 1 & 2). Unlike other commonly used nuclear stains such as propidium iodide or DRAQ7™, NucFix labeling is extremely stable, allowing the cells to be fixed and permeabilized without loss of fluorescence or dye transfer between cells.

Biotium also offers a selection of eight cytoplasm-staining Live-or-Dye™ viability stains spanning the fluorescence spectrum (catalog numbers 32002-32009), for maximal flexibility in multi-color analysis.

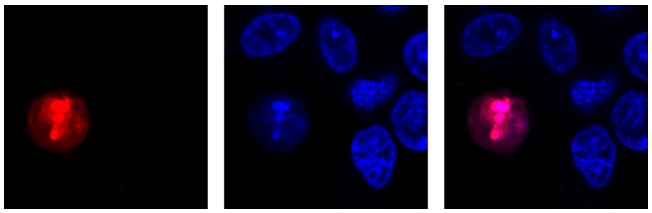

Figure 1. HeLa cells were treated with 5% ethanol for 10 minutes to kill a subset of the cells and then stained according to the product protocol with Live-or-Dye NucFix Dead Cell Stain (red). Cells were also stained with Hoechst (blue) to label the nuclei of both live and dead cells and imaged on a Zeiss LSM 700 confocal microscope (NucFix Red in the Cy3 channel, Hoechst in the DAPI channel). Only dead cells show bright, red nuclear staining with NucFix.

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.

1. 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 x 10^6 cells per staining reaction.
2. 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.
3. Pellet the desired number of cells by centrifugation at 350 x*g* for five minutes and gently pour off supernatant. For all subsequent steps, pellet cells by centrifugation after each incubation or wash.
4. Wash cells once in PBS, and resuspend in PBS at a concentration of 1 x 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 NucFix™ staining.
5. Aliquot cells into FACS tubes, 1 mL (1 x 10^6 cells) per tube.
6. Add 1 uL of NucFix Red Dead Cell Stain to 1 mL cells and immediately mix well.
7. Incubate for 30 minutes at room temperature or on ice, protected from light.
8. 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.
9. Stain for surface antigens:

   a. Add the appropriate primary antibodies to cells in PBS.
   b. Incubate for 15 minutes on ice or at room temperature in the dark.
   c. Wash cells twice with 1 mL PBS.
   d. If necessary, repeat steps a-c with the appropriate secondary antibodies.
   e. Proceed to step 10 for fixation. Otherwise, skip to step 13.
10. 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 on next page).

    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.
11. 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.

12. Perform intracellular staining:

    a. Resuspend cells in 100 uL 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 channel (i.e., PE-Texas Red®).

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

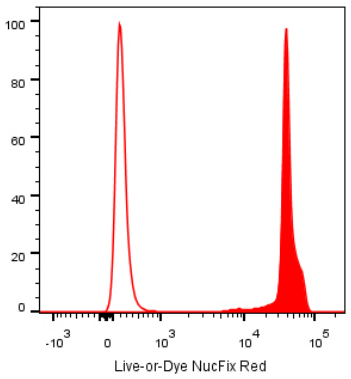

Figure 2. Live or heat-killed Jurkat cells were stained according to the product protocol with Live-or-Dye NucFix Red. Fluorescence was measured on a BD LSRII flow cytometer in the PE-Texas Red® channel. Heat-killed cells (solid peak) showed much higher fluorescence intensity compared to live cells (white peak), allowing the two populations to be clearly distinguished. Results are shown for unfixed cells; nearly identical histograms were observed after fixation and permeabilization according to the protocol. Fluorescence histograms were analyzed using FlowJo data analysis software.

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.

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 10%, incubate for 10 minutes, and wash once with PBS. Replace with PBS and proceed with the protocol.
3. Wash cells with PBS and replace media with PBS containing 1X NucFix Red 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 uL dye to 100 uL 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 and stain according to your standard protocols. Cells can also be co-stained with an appropriate dye such as DAPI (40043) or Hoechst (40046).
9. 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.

Related Products

Catalog number	Product
32002-T	Live-or-Dye™ 350/448 Fixable Viability Staining Kit, Trial Size
32003-T	Live-or-Dye™ 405/452 Fixable Viability Staining Kit, Trial Size
32009-T	Live-or-Dye™ 405/545 Fixable Viability Staining Kit, Trial Size
32004-T	Live-or-Dye™ 488/515 Fixable Viability Staining Kit, Trial Size
32005-T	Live-or-Dye™ 568/583 Fixable Viability Staining Kit, Trial Size
32006-T	Live-or-Dye™ 594/614 Fixable Viability Staining Kit, Trial Size
32007-T	Live-or-Dye™ 640/662 Fixable Viability Staining Kit, Trial Size
32008-T	Live-or-Dye™ 750/777 Fixable Viability Staining Kit, Trial Size
30068	ViaFluor™ 405-SE Cell Proliferation Kit
30050	ViaFluor™ CFSE Cell Proliferation Kit
30080	ViaFluor™ 568-SE Cell Proliferation Kit
23006	Flow Cytometry Fixation/Permeabilization Kit
22015	Fixation Buffer
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer
22002	TWEEN® 20
30069	AccuEasy™ Flow Cytometry Kit
22003	Mini Cell Scrapers
22014	Bovine Serum Albumin 30% solution
22010	10X Fish Gelatin Blocking Agent
30029	NucView™ 488 Caspase-3 Assay Kit for Live Cells
30065	Apoptosis and Necrosis Quantitation Kit Plus
29009	CF™405M Annexin V
29005	CF™488A Annexin V
40043	DAPI in H2O, 10 mg/mL
40046	Hoechst 33342, 10 mg/mL in H2O
23002	EverBrite™ Mounting Medium with DAPI
23004	EverBrite™ Hardset Mounting Medium with DAPI

Please visit our website at www.biotium.com for information on our life science research products, including primary antibodies, fluorescent CF™dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.