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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Product Information

Live-or-Dye™ Fixable Viability Staining Kits

Single-Color Kit Contents

Component	Trial size 50 labelings	Full size 200 labelings
Live-or-Dye™ Fixable Viability Dye	Component A 1 vial	Component A 4 vials
Anhydrous DMSO	99953 150 uL	99953-1 250 uL

Sampler Kit Contents

Component	32016 Standard Kit	32017 Spectral Kit
Live-or-Dye™ Fixable Viability Dyes 1 vial of each	32002A (350/448) 32009A (405/545) 32004A (488/515) 32005A (568/583) 32007A (640/662)	32002A (350/448) 32014A (375/600) 32012A (510/550) 32015A (615/740) 32013A (665/685)
Anhydrous DMSO	99938 500 uL	99938 500 uL

Storage and Handling

Store the kit at -20°C. Product is stable for at least 12 months from date of receipt when stored as recommended.

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 impermeant 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. 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, in order to prevent interference with immunostaining. Biotium offers a wide selection of Live-or-Dye™ viability stains spanning the fluorescence spectrum, for maximal flexibility in designing multi-color flow panels. We also offer two sampler kits, each containing five different dye colors, for excitation from all of the popular flow cytometry laser lines. The Live-or-Dye™ Sampler Kit, Standard (Cat. No. 32016) was designed for use on the most common flow cytometer laser and filter configurations, with dyes excitable by UV, violet, blue, yellow-green, and red lasers. The Live-or-Dye™ Sampler Kit, Spectral (Cat. No. 32017) was designed for use in spectral scanning flow cytometry. It contains dyes excitable by UV, violet, blue, and red lasers, all of which have been validated on the Cytek® Aurora spectral cytometer, and chosen for their ability to fill the gaps in many spectral flow panels.

We also offer Live-or-Dye NucFix™ Red (Cat. No. 32020), a fixable, nuclear-specific dead cell stain for flow cytometry or microscopy that is a superior alternative to non-fixable dyes like propidium iodide (PI).

Special Application Notes

- Live-or-Dye™ 350/448, 375/600, 510/550, 615/740, and 665/685 have been validated for use in spectral cytometry on the Cytek® Aurora.
- Live-or-Dye™ 488/515, 568/583, 594/614, and 640/662 have been validated for use in fluorescence microscopy.
- Other Live-or-Dye™ colors are also expected to work for microscopy, or for standard or spectral flow, if appropriate excitation sources, detection filters, and panel design are used. Live-or-Dye™ 350/448 is less photostable than the other dyes and not recommended for fluorescence microscopy.

Experimental Protocols

Dye Reconstitution

Remove one vial of dye and the anhydrous DMSO and bring to room temperature. Add 50 uL of anhydrous DMSO to the vial, vortexing 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 and protected from light at -20°C for at least 12 months.

Protocol 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 5 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 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 Live-or-Dye™ staining.
5. Aliquot cells into FACS tubes, with 1 mL containing 1 x 10^6 cells per tube.
6. Add 1 uL of Fixable Dead Cell Dye 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 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 Fixation Buffer (Cat. No. 22015), 2-4% formaldehyde, or your preferred fixation reagent for 20 minutes at room temperature.

    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 buffer (PBS + 1% serum, 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 Permeabilization Buffer (Cat. No. 22016), 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 buffer.

    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 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.

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. See “Special Application Notes” on page 1 for a list of dyes validated for microscopy.

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 coverslip.
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 a 1:1000 dilution of Fixable Dead Cell Dye. Alternatively, add the dye 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 with 0.1-0.5% Triton® X-100 for 5-10 minutes.
9. Proceed with the immunostain and/or cellular stain of your choice. Cells can also be stained with an appropriate DNA dye (see Related Products).
10. Wash cells once more in PBS.
11. Cells can be imaged immediately on the chamber slide or dish. Fixed cells can be mounted using antifade mounting medium, such as EverBrite™ Mounting Medium (see Related Products) if desired.

Table 1. Spectral Properties

Catalog No.	Product	Laser line (nm)	Detection channel	Ex/Em (nm)
32018, 32018-T	Live-or-Dye™ 330/410	355	BUV395	330/410
32002, 32002-T	Live-or-Dye™ 350/448	355	DAPI	355/450
32014, 32014-T	Live-or-Dye™ 375/600	355 or 405	Spectral scan	373/595
32003, 32003-T	Live-or-Dye™ 405/452	405	Pacific Blue™	416/452
32009, 32009-T	Live-or-Dye™ 405/545	405	AmCyan	413/547
32004, 32004-T	Live-or-Dye™ 488/515	488	FITC	490/516
32012, 32012-T	Live-or-Dye™ 510/550	488	Spectral scan	516/549
32005, 32005-T	Live-or-Dye™ 568/583	488 or 561	PE	562/584
32006, 32006-T	Live-or-Dye™ 594/614	488 or 561	PE-Texas Red®	593/615
32015, 32015-T	Live-or-Dye™ 615/740	633	Spectral scan	612/744
32007, 32007-T	Live-or-Dye™ 640/662	633 or 640	APC	642/663
32013, 32013-T	Live-or-Dye™ 665/685	633 or 640	Spectral scan	667/685
32008, 32008-T	Live-or-Dye™ 750/777	633 or 640	APC-Cy®7	755/779
32011, 32011-T	Live-or-Dye™ 787/808	785 or 808	APC-Cy®7 or IR840	783/808
32021, 32021-T	Live-or-Dye™ 820/835	808	IR840	822/835
32022, 32022-T	Live-or-Dye™ 850/870	808	IR885	852/870

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF® Dye antibody conjugates, Mix-n-Stain™ antibody labeling kits, apoptosis reagents including Annexin V conjugates, and other fluorescent probes and tools for cell biology research.

Related Products

Cat. No.	Product
32010	Live-or-Dye NucFix™ Red
90082	Anhydrous DMSO
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22015	Fixation Buffer
22016	Permeabilization Buffer
22003	Mini Cell Scrapers
23006	Flow Cytometry Fixation/Permeabilization Kit
30050... 30139	ViaFluor® SE Cell Proliferation Kits
41033... 41040	NucSpot® Nuclear Stains
40061	RedDot™2 Far-Red Nuclear Stain, 200X in DMSO
40009... 40043	DAPI
40044-40047	Hoechst
10402... 10408	NucView® Caspase-3 Enzyme Substrates
30030... 30073	Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate & Annexin V
30072	NucView® 488 & RedDot™2 Apoptosis & Necrosis Kit
30062	NucView® 488 & MitoView™ 633 Apoptosis Assay Kit
70054... 70082	MitoView™ Mitochondrial Dyes
29001... 29126	Annexin V Conjugates
29003R... 29085R	Annexin V CF® Dye Conjugates, Azide-Free, Lyophilized
23001, 23002	EverBrite™ Mounting Medium (with or without DAPI)
23003... 23016	EverBrite™ Hardset Mounting Medium (with or without DAPI or NucSpot® 640)
23017-23019	EverBrite TrueBlack® Hardset Mounting Medium (with or without DAPI or NucSpot® 640)
23008, 23009	Drop-n-Stain EverBrite™ Mounting Medium (with or without DAPI)
23005	CoverGrip™ Coverslip Sealant