ab314684 – Fixable Viability Staining Assay Kit (Ex/Em = 665/685 nm)

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab314684

Storage and Stability:

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.
Stock solutions may be prepared in DMSO. Solutions can be aliquoted and stored with desiccant and protected from light at -20°C, for at least 1 year.

Materials Supplied

Item	50 Tests	200 Tests	Storage Condition
665/685 Fixable Viability Dye	1 vial	4 vials	-20°C
Anhydrous DMSO	150 uL	250 uL	-20°C

Experimental Protocols

Dye Reconstruction

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 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 year.

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 (ab285410) and resuspend in PBS at 1 x 10^6 cells/mL.

Δ 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 Fixable Viability Dye.*

5. Aliquot cells into FACS tubes, 1 mL (1 x 10^6 cells) per tube.

6. Add 1 uL of Fixable Viability 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.

Δ 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 (ab314680), 2-4% formaldehyde, or your preferred fixation reagent for 20 minutes at room temperature.

Δ Note: *For intracellular staining, other fixation methods may be optimal for specific antibodies. Because Fixable Viability Dye 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 (ab314681), PBS + 0.1% Triton® X-100 (ab286840), 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).

Δ 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.

1. Grow cells in culture as required for your experiment. For adherent cells, staining can be done in a chamber slide, in a multi-well 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 Viability Dye. 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.

Δ 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 (ab286840), 5-10 min.

9. Proceed with the immunostain and/or cellular stain of your choice. Cells can also be stained with an appropriate DNA dye such as DAPI Staining Solution (ab228549) or Hoechst 33342 Staining Dye Solution (ab228551).

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 Anti-Fade Fluorescence Mounting Medium (ab104135) if desired.

Spectral properties of Fixable Viability Dye (665/685)

Item	Laser Line (nm)	Detection Channel	Ex/Em (nm)
665/685 Fixable Viability Dye	633 or 640	Spectral scan	667/685

For technical support contact information, visit: www.abcam.com/contactus

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