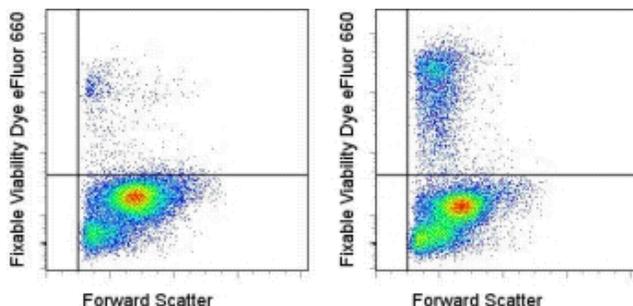


## Fixable Viability Dye eFluor® 660

Catalog Number: 65-0864

GPR: General Purpose Reagents. For Laboratory Use.



BALB/c thymocytes were uncultured (left) or cultured overnight at 37°C (right) and then stained with Fixable Viability Dye eFluor® 660. Total cells were used for analysis.

### Product Information

**Contents:** Fixable Viability Dye eFluor® 660

**REF** Catalog Number: 65-0864

**Formulation:** Dye in DMSO, pre-diluted to test size

**Temperature Limitation:** Store at less than or equal to -70°C. Protect from light and moisture.

**LOT** Batch Code: Refer to Vial

**Use By:** Refer to Vial

### Description

Fixable Viability Dye eFluor® 660 is a viability dye that can be used to irreversibly label dead cells prior to cryopreservation, fixation and/or permeabilization procedures. Unlike 7-AAD and propidium iodide, cells labeled with Fixable Viability Dyes can be washed, fixed, permeabilized, and stained for intracellular antigens without any loss of staining intensity of the dead cells. Thus, using Fixable Viability Dyes allows dead cells to be excluded from analysis when intracellular targets are being studied. These dyes may be used to label cells from all species.

Fixable Viability Dye eFluor® 660 can be excited by the red (633 nm) laser line and has a peak emission of 660 nm that can be detected using a 660/20 band pass filter (equivalent to APC or Alexa Fluor® 647). Please make sure that your instrument is capable of detecting this dye. For compensation, it is recommended to use a sample of the cells of interest stained with the Fixable Viability Dye. If the percentage of dead cells is expected to be less than 5%, then it is recommended to take a small aliquot of cells and heat them at 65°C for 1 minute then immediately place on ice for 1 minute. After this treatment, the heat-killed cells can be combined 1:1 with live cells and then stained with the Fixable Viability Dye.

Fixable Viability Dye eFluor® 660 is supplied as a pre-diluted solution prepared in high-quality, anhydrous DMSO. It should be protected from light and moisture. Store at -80°C with desiccant. It may be freeze-thawed up to 20 times. Allow the vial to equilibrate to room temperature before opening.

### Applications Reported

Fixable Viability Dye eFluor® 660 has been reported for use in flow cytometric analysis.

### Applications Tested

Fixable Viability Dye eFluor® 660 has been pre-titrated and tested by flow cytometric analysis of mouse thymocytes. Fixable Viability Dyes are fully compatible with the IC Fixation and Permeabilization Buffers and the Foxp3 Buffer Set. This can be used at 1 µL/mL of cells resuspended at 1-10x10<sup>6</sup> cells per mL in azide-free and serum/protein-free PBS. It is recommended that the concentration used be determined by each investigator for optimal performance in the assay of interest.

### Special Notes

Staining with Fixable Viability Dye eFluor® 660 may be done before or after surface staining. Cells may be cryopreserved after staining with Fixable Viability Dye eFluor® 660 with no adverse effects on the staining intensity of dead cells after thawing.

### Related Products

00-5523 Foxp3 / Transcription Factor Staining Buffer Set

65-0863 Fixable Viability Dye eFluor® 450

65-0865 Fixable Viability Dye eFluor® 780

65-0866 Fixable Viability Dye eFluor® 506

88-8823 Fixation & Permeabilization Buffers

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## Fixable Viability Dye Cell Staining Protocol

Research Use Only

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### Protocol: Fixable Viability Dye Cell Staining

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#### Experimental Procedure

Allow vial of Fixable Viability Dye to equilibrate to room temperature before opening. Staining with Fixable Viability Dye must be done in azide-free and serum/protein-free PBS. For consistent staining of cells, do not stain cells in less than 0.5 mL.

1. Prepare cells as desired.
2. Wash cells 2 times in azide-free and serum/protein-free PBS.
3. Resuspend cells at  $1-10 \times 10^6$ /mL in azide-free and serum/protein-free PBS.
4. Add 1  $\mu$ L of Fixable Viability Dye per 1 mL of cells and vortex immediately.
5. Incubate for 30 min at 2-8°C, protect from light.
6. Wash cells 1-2 times with flow staining buffer or equivalent.
7. Fix and/or permeabilize cells as desired.

*Note: Cells may be stained with Fixable Viability Dyes before or after surface staining. After staining with Fixable Viability Dyes, cells may also be cryopreserved for analysis at a later time. It is recommended that each investigator determine the optimal concentration for the assay of interest.*