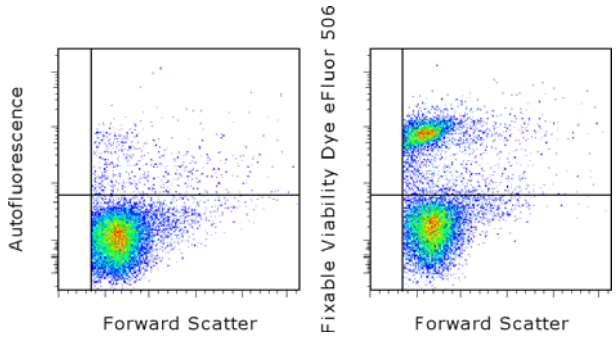
# eBioscience™ Fixable Viability Dye eFluor™ 506

**Catalog Number:** 65-0866
**Also known as:** FVD eFluor® 506
**For Research Use Only. Not for use in diagnostic procedures.**




Staining of C57Bl/6 thymocytes cultured overnight with staining buffer (autofluorescence) (left) or Fixable Viability Dye eFluor® 506 (right). Total cells were used for analysis.

## Product Information

**Contents:** eBioscience™ Fixable Viability Dye eFluor™ 506
REF **Catalog Number:** 65-0866

**Formulation:** 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® 506 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, permabilized, 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. Fixable Viability Dyes may be used to label cells from all species.

Fixable Viability Dye eFluor® 506 can be excited by the violet (405 nm) laser line and has a peak emission of 506 nm that can be detected using a 510/50 band pass filter (equivalent to AmCyan). 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® 506 is supplied as a pre-diluted solution prepared in high-quality, anhydrous DMSO. It should be protected from light and moisture. Store at less than or equal to -70°C with dessicant. It may be freeze-thawed up to 20 times. Allow vial to equilibrate to room temperature before opening.

## Applications Reported

Fixable Viability Dye eFluor® 506 has been reported for use in flow cytometric analysis.

## Applications Tested

Fixable Viability Dye eFluor® 506 has been pre-titrated and tested by flow cytometric analysis of mouse thymocytes. Fixable Viability Dyes are fully compatible with both IC Fixation and Permeabilization Buffers and the Foxp3/Transcription Factor Staining Buffer Set. This can be used at 1 µL/mL of cells resuspended at 1-10x10e6 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.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14 March 2017 Rev. 10

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**Special Notes**
Staining with Fixable Viability Dye eFluor® 506 may be done before or after surface staining. Cells may be cryopreserved after staining with Fixable Viability Dye eFluor® 506 with no adverse effect on staining intensity of dead cells after thawing.

**References**
Berga-Bolaños R, Sharma A, Steinke FC, Pyaram K, Kim YH, Sultana DA, Fang JX, Chang CH, Xue HH, Heller NM, Sen JM. ß-Catenin is required for the differentiation of iNKT2 and iNKT17 cells that augment IL-25-dependent lung inflammation. BMC Immunol. 2015 Oct 19;16:62. (**FVD eFluor 506**, FC, PubMed)

Kurtulus S, Sakuishi K, Ngiow SF, Joller N, Tan DJ, Teng MW, Smyth MJ, Kuchroo VK, Anderson AC. TIGIT predominantly regulates the immune response via regulatory T cells. J Clin Invest. 2015 Nov 2;125(11):4053-62. (**FVD eFluor 506**, FC, PubMed)

**Related Products**
00-5523 eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set
65-0863 eBioscience™ Fixable Viability Dye eFluor™ 450
65-0864 eBioscience™ Fixable Viability Dye eFluor™ 660
65-0865 eBioscience™ Fixable Viability Dye eFluor™ 780
65-0867 eBioscience™ Fixable Viability Dye eFluor™ 520
65-0868 eBioscience™ Fixable Viability Dye eFluor™ 455UV
65-2860 eBioscience™ Fixable Viability Dye eFluor™ 506/780 Sample Pack
88-8824 eBioscience™ Intracellular Fixation & Permeabilization Buffer Set


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# Fixable Viability Dye Cell Staining Protocol

## Introduction

Fixable Viability Dyes (FVD) are viability dyes 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 FVD can be washed, fixed, permeabilized, and stained for intracellular antigens without any loss of staining intensity of the dead cells. Thus, using FVD allows dead cells to be excluded from analysis when intracellular targets are being studied. FVD may be used to label cells from all species.

The following table summarizes the available FVD along with their optical properties:

**Table of Fixable Viability Dyes**

| Catalog Number | Format | Excitation source (nm) | Emission (nm) |
|---|---|---|---|
| 65-0868 | Fixable Viability Dye eFluor™ 455UV | 350 | 455 |
| 65-0863 | Fixable Viability Dye eFluor™ 450 | 405 | 450 |
| 65-0866 | Fixable Viability Dye eFluor™ 506 | 405 | 506 |
| 65-0867 | Fixable Viability Dye eFluor™ 520 | 488 | 522 |
| 65-0864 | Fixable Viability Dye eFluor™ 660 | 633 | 660 |
| 65-0865 | Fixable Viability Dye eFluor™ 780 | 633 | 780 |
| 65-2860 | Fixable Viability Dye eFluor™ 506/780 Sample Pack | - | - |

**Table 1: Table of Fixable Viability Dyes**

## General Notes

### Best practices when using Fixable Viability Dyes

1. FVD are supplied as a pre-diluted solutions prepared in high-quality, anhydrous DMSO. They should be protected from light and moisture. Store at less than or equal to -70°C with desiccant. They may be freeze-thawed up to 20 times.
2. Allow vial of FVD to equilibrate to room temperature before opening.
3. For the brightest staining, it is best to stain with FVD in azide- and protein-free phosphate-buffered saline (PBS).
4. Cells may be stained with FVD before or after surface staining. After staining with FVD, 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.
5. Although FVD may often be used in combination with fixation, permeabilization and intracellular staining, FVD may also be used experiments using live, unfixed cells.
6. For compensation, it is recommended to use a sample of the cells of interest stained with the FVD only. 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 FVD.

### Alternative staining procedures (Protocols C, D, and E)

1. Protocols C, D and E are modifications for ease-of-use which may result in reduced staining intensity of the dead cells. These alternative staining protocols should be avoided if maximum staining intensity is desired. It is recommended that each investigator determine whether these protocol modifications provide sufficient staining intensity of dead cells.
2. It is possible to stain un-lysed, whole blood with FVD. See Protocol C below for details.
3. It is possible to stain in azide-free, but protein-containing PBS. This method may result in a small reduction in the staining intensity of the dead cell population. See Protocol D below for details.
4. It is possible to stain in azide- and protein-containing PBS, such as Flow Cytometry Staining Buffer (Cat. No. 00-4222). This method may result in a significant decrease in the staining intensity of the dead cell population and/or an increase in background staining of the live cell population. See Protocol D below for details.
5. It is possible to add the FVD to an antibody cocktail before addition to the cells. The FVD should spend as little time as possible in the cocktail prior to staining. It is best to use azide-free, protein containing buffer for dilution of the antibody cocktail and FVD. See Protocol E below for details.

## Protocol A: Standard staining in tubes

### Materials

- Phosphate-buffered saline (PBS), azide- and protein-free
- Flow Cytometry Staining Buffer (Cat. No. 00-4222)
- 12x75 mm round bottom test tubes

### Experimental Procedure

1. Prepare cells in 12x75 mm tubes.
2. Wash cells 2 times in azide-free and protein-free PBS.
3. Resuspend cells at 1-10x$10^6$/mL in azide-free and serum/protein-free PBS.

*Note: For consistent staining of cells, we do not recommend staining in less than 0.5 mL.*

4. Add 1 µL of FVD per 1 mL of cells and vortex immediately.
5. Incubate for 30 minutes at 2-8°C, protect from light.
6. Wash cells 1-2 times with Flow Cytometry Staining buffer or equivalent.
7. Continue with experiment, as desired.

## Protocol B: Staining in 96-well plates

### Materials

- Phosphate-buffered saline (PBS), azide- and protein-free
- Flow Cytometry Staining Buffer ( Cat. No. 00-4222)
- 96-well assay plates

### Experimental Procedure

1. Prepare cells as desired in 96-well plates.
2. Wash cells 2 times in azide-free and serum/protein-free PBS. Completely decant supernatant.
3. Prepare a working solution of the FVD by diluting it 1:1000, in azide- and serum/protein-free PBS. Make enough for 100 µL/well. For example, if you need enough for 96 wells, add 10 µL of FVD to 10 mL of PBS.
4. Add 100 µL of the working solution of the FVD to each well and mix immediately by pipetting or gentle vortexing.
5. Incubate for 30 minutes at 2-8°C, protect from light.
6. Wash cells 1-2 times with Flow Cytometry Staining buffer or equivalent.
7. Continue with experiment, as desired.

## Protocol C: Staining with FVD in un-lysed whole blood

### Materials

- Phosphate-buffered saline (PBS), azide- and protein-free
- Flow Cytometry Staining Buffer (Cat. No. 00-4222)
- Red blood cell lysis buffer, such as 1X RBC Lysis Buffer (Cat. No. 00-4333), 10X RBC Lysis Buffer (Multi-species) (Cat. No. 00-4300), or 1-step Fix/Lyse Solution (10X) (Cat. No. 00-5333)
- 12x75 mm round bottom test tubes

## Experimental Procedure

1. Add un-lysed whole blood to 12x75 mm tubes.
2. Add 1 µL of FVD per 100 µL of whole blood.
3. Add other surface staining antibodies after addition of the FVD.

*Note: Alternatively, FVD may be added directly to the surface staining antibody cocktail at 1 µL per sample to be stained. This cocktail should be made just prior to addition to whole blood samples. See Protocol E below for details.*

4. Incubate for 30 minutes at 2-8°C, protect from light.
5. Wash samples 1-2 times with Flow Cytometry Staining buffer.
6. Lyse red blood cells and continue with experiment, as desired.

## Protocol D: Staining with FVD in azide- and/or protein-containing staining buffers

### Materials

- Flow Cytometry Staining Buffer (Cat. No. 00-4222)
- 12x75 mm round bottom test tubes

## Experimental Procedure

1. Prepare cells in 12x75 mm tubes at 1-10x$10^6$/mL in Flow Cytometry Staining buffer.
2. Add 1 µL of FVD per 1 mL of cells and vortex immediately.
3. Incubate for 30 minutes at 2-8°C, protect from light.
4. Wash cells 1-2 times with Flow Cytometry Staining buffer.
5. Continue with experiment, as desired.

## Protocol E: Staining with FVD in an antibody cocktail

### Materials

- Phosphate-buffered saline (PBS), azide- and protein-free
- Flow Cytometry Staining Buffer (Cat. No. 00-4222)
- 12x75 mm round bottom test tubes

## Experimental Procedure

1. Prepare cells in 12x75 mm tubes and resuspend at 1-10x$10^6$ in 100 µL of azide- and serum/protein free PBS, as described in Protocol A, for maximum brightness.

*Note: If maximal brightness is not critical, cells may be resuspended in Flow Cytometry Staining buffer (as described in Protocol D).*

2. Prepare desired antibody cocktail in Flow Cytometry Staining buffer.
3. Immediately prior to addition to cells, add FVD to antibody cocktail at 0.5-1 µL per sample to be stained. Mix well.
4. Add FVD/antibody cocktail to cell samples.
5. Incubate for 30 minutes at 2-8°C, protect from light.
6. Wash cells 1-2 times with Flow Cytometry Staining buffer.
7. Continue with experiment, as desired.

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