# Live or Dead™ Fixable Dead Cell Staining Kit *Blue Fluorescence with 405 nm Excitation*

Catalog number: 22500
Unit size: 200 Tests

| Component | Storage | Amount (Cat No. 22500) |
|---|---|---|
| Component A: Stain It™ Violet 450 | Freeze (< -15 °C), Minimize light exposure | 1 vial |
| Component B: DMSO | Freeze (< -15 °C) | 1 vial (200 µL) |

## OVERVIEW

Our Live or Dead™ Fixable Dead Cell Staining Kits are a set of tools for labeling cells for fluorescence microscopic investigations of cellular functions. The effective labeling of cells provides a powerful method for studying cellular events in a spatial and temporal context. This particular kit is designed to uniformly label fixed mammalian cells in blue fluorescence for flow cytometry applications with violet laser excitation. The kit uses a proprietary blue fluorescent dye that is more fluorescent upon bonding to cellular components. The fluorescent dye used in the kit is well excited with the violet laser (405 nm excitation) to fluorescence at 460 nm. The kit provides all the essential components with an optimized cell-labeling protocol. It is an excellent tool for preserving of fluorescent images of particular cells, and can also be used for fluorescence flow cytometry applications.

## AT A GLANCE

**Protocol Summary**

1. Prepare samples in HHBS (0.5 mL/assay)
2. Replace with HHBS
3. Add Stain It™ Violet 450 to the cell suspension
4. Stain the cells at room temperature or 37°C for 20 - 60 minutes
5. Wash the cells
6. Fix the cells (optional)
7. Examine the sample with flow cytometer and/or fluorescence microscope using the appropriate Excitation/Emission filter

**Important** Thaw all the components at room temperature before starting the experiment.

## KEY PARAMETERS

**Fluorescence microscope**

| | |
|---|---|
| Excitation | 410 nm |
| Emission | 450 nm |
| Recommended plate | Black wall/clear bottom |

**Flow cytometer**

| | |
|---|---|
| Excitation | |
| Emission | |
| Instrument specification(s) | see Table 1 |

## CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

## PREPARATION OF STOCK SOLUTIONS

*Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles*

**Stain It™ Violet 450 stock solution (500X)**

Add 200 µL DMSO (Component B) into the vial of Stain It™ Violet 450 (Component A) to make 500X Stain It™ Violet 450 stock solution.

## SAMPLE EXPERIMENTAL PROTOCOL

**Table 1.** Fluorescence spectra properties and suggested excitation laser for flow cytometry analysis

| Cat. # | Description | Ex (nm) | Em (nm) | Excitation Source |
|---|---|---|---|---|
| 22500 | Blue Fluorescence with 405 nm Excitation | 410 | 450 | 405 nm |
| 22501 | Green Fluorescence with 405 nm Excitation | 408 | 512 | 405 nm |
| 22502 | Orange Fluorescence with 405 nm Excitation | 398 | 550 | 405 nm |
| 22599 | Red Fluorescence Optimized for Flow Cytometry | 523 | 617 | 488 nm |
| 22600 | Blue Fluorescence | 353 | 442 | 335 nm |
| 22601 | Green Fluorescence | 498 | 521 | 488 nm |
| 22602 | Orange Fluorescence | 547 | 573 | 561 nm or 488 nm |
| 22603 | Red Fluorescence | 583 | 603 | 561 nm |
| 22604 | Deep Red Fluorescence | 649 | 660 | 633 nm |
| 22605 | Near Infrared Fluorescence | 749 | 775 | 633 nm |

1. Prepare cells using 1X Hanks and 20 mM Hepes buffer (HHBS) or sodium azide-free and serum/protein-free buffer of your choice.
2. Wash cells once with HHBS or the azide- and serum/protein-free buffer of your choice.
3. Resuspend cells at 5 - 10 × $10^6$/mL in HHBS or in the azide- and serum/protein-free buffer of your choice.
4. Add 1 µL of 500X Stain It™ Violet 450 stock solution to 0.5 mL of cells/assay and mix it well.
5. Incubate at room temperature or 37°C, 5% $CO_2$ incubator for 20 - 60 minutes, protected from light.

   **Note:** The optimal stain concentrations and incubation time should be experimentally determined for different cell lines.
6. Wash cells twice and resuspend cells with HHBS or the buffer of your choice.
7. Fix cells as desired (optional).
8. Analyze cells with flow cytometer and/or fluorescence microscope using the appropriate Excitation/Emission filter (see Table 1).


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## EXAMPLE DATA ANALYSIS AND FIGURES

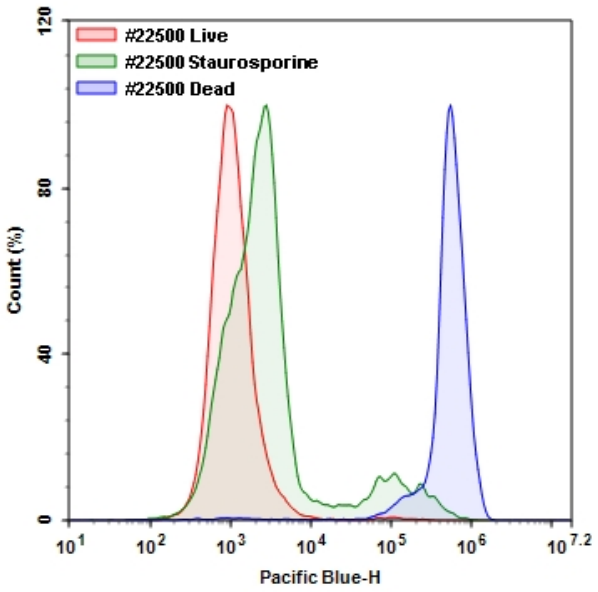



**Figure 1.** Detection of Jurkat cell viability by Live or Dead™ Fixable Dead Cell Staining Kits (Cat#22500). Jurkat cells were treated and stained with Stain It™ Violet 450, and then fixed in 3.7% formaldehyde and analyzed by flow cytometry. Live (Red), staurosporine treated (Green) and heat-treated (Blue) cells were distinguished with Pacific Blue channel.

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