Live or Dead™ Fixable Dead Cell Staining Kit *Blue Fluorescence*

Catalog number: 22600
Unit size: 200 Tests

Component	Storage	Amount
Component A: Stain It™ Blue	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 cell 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 long term microscopic examination. 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 quite photostable so that the images can be repeatedly examined. 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 microscope demonstrations.

AT A GLANCE

Protocol summary

1. Prepare samples in HHBS (0.5 mL/assay)
2. Replace with HHBS
3. Add Stain It™ Blue 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

Instrument:	Fluorescence microscope
Excitation:	353 nm
Emission:	442 nm
Recommended plate:	Black wall/clear bottom

Instrument:	Flow cytometer
Excitation:	355 nm or 405 nm laser
Emission:	450/40 nm filter
Instrument specification(s):	Pacific Blue channel

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.

1. Stain It™ Blue stock solution (500X):
Add 200 µL DMSO (Component B) into the vial of Stain It™ Blue (Component A) to have 500X Stain It™ Blue stock solution.

PREPARATION OF CELL SAMPLES

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

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™ Blue 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).

EXAMPLE DATA ANALYSIS AND FIGURES

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

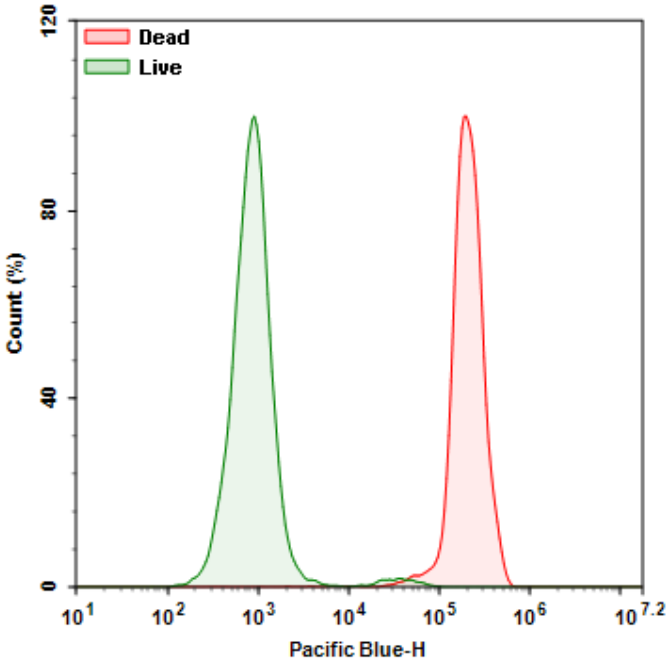

Figure 1. Detection of Jurkat cell viability by Live or Dead™ Fixable Dead Cell Staining Kits (Cat#22600). Jurkat cells were treated and stained with Stain It™ Blue . The cells were fixed in 3.7% formaldehyde and analyzed by flow cytometry as described above. Live (Green) and Dead (heat-treated, Red) cells were distinguished with Pacific Blue channel. The live cell population is easily distinguished from the dead cell population, and nearly identical results were obtained using unfixed cells.