Live or Dead™ Fixable Dead Cell Staining Kit *Red Fluorescence Optimized for Flow Cytometry*

Catalog number: 22599
Unit size: 200 Tests

Component	Storage	Amount
Component A: Stain It™ Red 620	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: DMSO	Freeze (< -15 °C)	1 vial (200 uL)

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 red fluorescence for flow cytometry applications with blue or green laser excitation. The kit uses a proprietary red fluorescent dye that is more fluorescent upon binding to cellular components. The fluorescent dye used in the kit is well excited with the blue (488 nm) and green lasers (532 nm) to fluorescence at 620 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™ Red 620 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

Flow cytometer

Excitation	488 nm or 532 nm laser
Emission	610/20 nm filter

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™ Red 620 stock solution (500X)
Add 200 µL DMSO (Component B) into the vial of Stain It™ Red 620 (Component A) to have 500X Stain It™ Red 620 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™ Red 620 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

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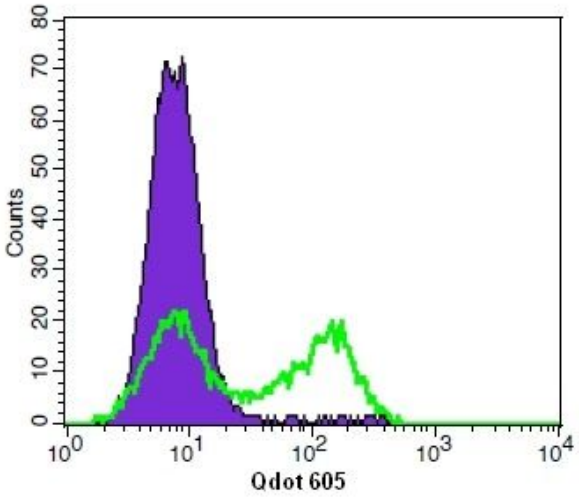

Figure 1. Detection of Jurkat cell viability by Live or Dead™ Fixable Dead Cell Staining Kits 22599. Jurkat cells were treated and stained with Stain IT™ Red 620. Live (violet solid peak) and staurosporine treated (green line) cells were measured with Qdot®605 Channel. The live cell population is easily distinguished from the dead cell population.

DISCLAIMER

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