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

Catalog number: 22598
Unit size: 200 Tests

Component	Storage	Amount (Cat No. 22598)
Component A: Stain It™ IR fluorescence	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 infrared fluorescence for flow cytometry applications with IR laser excitation. The kit uses a proprietary IR fluorescent dye that is more fluorescent upon binding to cellular components. The fluorescent dye used in the kit is well excited with the IR laser (808 nm) and emits fluorescence at 878 nm (885/40 bandpass filter). The kit provides all the essential components with an optimized cell-labeling protocol. It is an excellent tool for the discrimination of live and dead cell populations by flow cytometry.

AT A GLANCE

Protocol Summary

1. Prepare samples in HHBS buffer (0.5 mL/assay).
2. Wash cells and replace with fresh HHBS buffer.
3. Add Stain It™ IR fluorescence 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 a flow cytometer using an 808 nm laser with an 885/40 nm or IR channel emission filter.

Important Note

Before starting the experiment, thaw all the components at room temperature.

KEY PARAMETERS

Flow cytometer

Emission	885/40 or IR Channel
Excitation	808 nm laser

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™ IR fluorescence stock solution (500X)

1. Add 200 µL of DMSO (Component B) to the vial of Stain It™ IR fluorescence (Component A) to make a 500X Stain It™ IR fluorescence stock solution.

SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare cells using 1X Hanks and 20 mM Hepes buffer (HHBS) or a sodium azide-free and serum/protein-free buffer of your choice.
2. Wash the cells once with HHBS or the azide- and serum/protein-free buffer of your choice.
3. Resuspend the 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™ IR fluorescence stock solution to 0.5 mL of cells/assay and mix 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 the cells twice and resuspend cells with HHBS or a buffer of your choice.
7. Fix cells as desired (optional).
8. Analyze cells with a flow cytometer using the appropriate excitation/emission specifications found in the 'Key Parameters' section.

DISCLAIMER

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