Live or Dead™ Fixable Dead Cell Staining Kits

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: See Table 1 (200 Assays)	Keep in freezer Protect from moisture and light	Flow cytometer

Introduction

Our Cell Live or Dead™ Fixable Dead Cell Staining Kits are a set of tools used to evaluate the viability of mammalian cells by flow cytometry. The fluorescent dyes provided in the kits are retained in cells by reacting with cellular components. For viable cells, only the cell-surface amines are available to react with the dyes while for the necrotic cells or the other cells with compromised membranes, the reactive dyes react with cell surface amines and intracellular amines, resulting in more intense fluorescent staining. The difference in fluorescence intensity between the live and dead cell populations is ~100-500 folds and can be completely preserved after fixation. Approximate fluorescence excitation and emission maxima are listed in Table 1.

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

Cat. #	Product Name	Ex (nm)	Em (nm)	Excitation Source
22500	Live or Dead™ Fixable Dead Cell Staining Kit *Blue Fluorescence with 405 nm Excitation*	410	450	405nm
22501	Live or Dead™ Fixable Dead Cell Staining Kit *Green Fluorescence with 405 nm Excitation*	408	512	405nm
22502	Live or Dead™ Fixable Dead Cell Staining Kit *Orange Fluorescence with 405 nm Excitation*	398	550	405nm
22599	Live or Dead™ Fixable Dead Cell Staining Kit *Red Fluorescence Optimized for Flow Cytometry*	523	617	488nm
22600	Live or Dead™ Fixable Dead Cell Staining Kit *Blue Fluorescence*	353	442	335 nm
22601	Live or Dead™ Fixable Dead Cell Staining Kit *Green Fluorescence*	498	521	488nm
22602	Live or Dead™ Fixable Dead Cell Staining Kit *Orange Fluorescence*	547	573	561nm or 488nm
22603	Live or Dead™ Fixable Dead Cell Staining Kit *Red Fluorescence*	583	603	561nm
22604	Live or Dead™ Fixable Dead Cell Staining Kit *Deep Red Fluorescence*	649	660	633nm

Kit Components

Components	Amount
Component A: Stain It™ Reactive Dye	1 vial
Component B: DMSO	1 vial (200 µL)

Assay Protocol

Brief Summary

Prepare samples (0.5 mL /assay) → Replace with HH buffer → Add Stain It™ Dye to the cell suspension → Stain the cells at room temperature or 37 °C for 20-60 minutes → Wash the cells → Fix the cells (optional) → Examine the sample with flow cytometer

Note: Thaw all the components at room temperature before opening.

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1) Add 200 µL DMSO (Component B) into the vial of Stain It™ Reactive Dye (Component A) to have 500X DMSO stock solution.
Note: The unused Stain It™ dye stock solution should be divided into single use aliquots and stored at -20 °C. Avoid repeated freeze/thaw cycles.
2) Prepare cells for flow cytometry staining using 1X Hanks and 20 mM Hepes buffer (HHBS) or sodium azide-free and serum /protein-free buffer of your choice.
3) Wash cells once with HHBS or the azide- and serum /protein-free buffer of your choice.
4) Resuspend cells at 5-10×10^6 /mL in HHBS or in the azide- and serum /protein-free buffer of your choice.
5) Add 1µL of Stain It™ Reactive Dye stock solution (from Step 1) to 0.5mL of cells/assay and mix it well.
6) Incubate for 20 to 60 min at room temperature or 37 °C, 5% CO_2 incubator, protected from light.
Note: The optimal stain concentrations and incubation time should be experimentally determined for different cell lines.
7) Wash cells twice and resuspend cells with HHBS or the buffer of your choice.
8) Fix cells as desired (optional).
9) Analyze cells with flow cytometer.

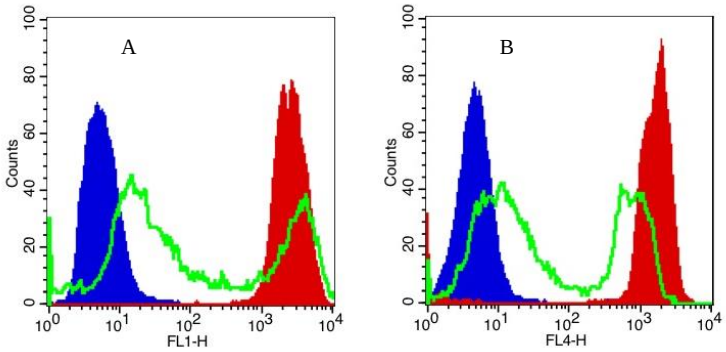

Figure 1. Detection of Jurkat cell viability by Live or Dead™ Fixable Dead Cell Staining Kits 22601 and 22604. Jurkat cells were treated and stained with Stain IT™ Green (22601, Panel A) and Stain IT™ Deep Red (22604, Panel B). The cells were fixed in 3.7% formaldehyde and analyzed by flow cytometry as described above. Live (Blue solid peak), staurosporine treated (green line) and heat-treated (red solid peak) cells were distinguished with Ex/Em = 488 nm and 520 nm (FL1) channel (panel A) and 633 nm/660 nm (FL4) channel (Panel B). The live cell population is easily distinguished from the dead cell population, and nearly identical results were obtained using unfixed cells.

References

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5. Giuliano KA. (2007) Optimizing the integration of immunoreagents and fluorescent probes for multiplexed high content screening assays. Methods Mol Biol, 356, 189.

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