Live or Dead™ Cell Viability Assay Kit *Red/Blue Dual Fluorescence*

Catalog number: 22788
Unit size: 200 Tests

Component	Storage	Amount (Cat No. 22788)
Component A: 200X Cellbrite™ Red	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component B: Assay Buffer	Freeze (< -15 °C)	50 mL
Component C: 200X Nuclear Blue™ DCS1	Freeze (< -15 °C), Minimize light exposure	1 Vial

OVERVIEW

This Live or Dead™ Cell Viability Assay Kit uses two fluorescent indicators: Cellbrite™ Red (Ex/Em = 613/631 nm) for labeling viable cells and a cell-impermeable DNA-binding dye Nuclear Blue™ DCS1 (Ex/Em = 360/450 nm) for labeling dead cells with damaged membranes. Cells grown in black-wall plates can be stained and quantified in less than two hours. The assay is more robust and accurate than the other viability assays. It can be readily adapted for a wide variety of fluorescence platforms such as microplate assays, fluorescence microscopes, and flow cytometry. The kit provides all the essential components with an optimized assay protocol. It is suitable for both proliferating and non-proliferating cells (either suspension or adherent cells).

AT A GLANCE

Protocol Summary

1. Prepare cells with test compounds
2. Add dye-working solution
3. Incubate at room temperature or 37°C for 30 minutes to 1 hour
4. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 610/650 nm (Cutoff = 630 nm, Red) and Ex/Em = 360/450 nm (Cutoff =420 nm, Blue) or fluorescence microscope with Cy5 channel (live) and DAPI channel (dead)

Important

Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microscope

Emission	Cy5 filter (alive), DAPI filter (dead)
Excitation	Cy5 filter (alive), DAPI filter (dead)
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Cutoff	630, 420 nm
Emission	650, 450 nm
Excitation	610, 360 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode

CELL PREPARATION

For guidelines on cell sample preparation, please visit:

https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF WORKING SOLUTION

Add 5 µL of 200X Cellbrite™ Red (Component A) and 5 µL of 200X Nuclear Blue™ DCS1 (Component C) into 1 mL of Assay Buffer (Component B) and mix well to make dye-working solution. This dye-working solution is stable for at least 1 hour at room temperature.

Note: As the optimal staining conditions may vary depending on different cell types, it's recommended to determine the appropriate concentration of Component A and C individually.

SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare cells according to the standard protocol. *Note:* We treated HeLa cells with staurosporine (SS) for 4 hours at 37°C to induce cell apoptosis. See Figure 1 for details.
2. Replace growth medium with 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of dye-working solution.
3. Incubate the dye-working solution plate at room temperature or 37°C for 30 minutes to 1 hour, protected from light.
4. Wash cells with HHBS, PBS or buffer of your choice twice.
5. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Assay Buffer (Component B) into the cells.
6. Monitor the fluorescence signal under a fluorescence microscope with Texas Red or Cy5 filter for live cells, and DAPI filter for dead cells. The fluorescence intensity can also be analyzed with a fluorescence microplate reader (bottom read mode) at Ex/Em = 610/650 nm (Cutoff = 630 nm, Red) and Ex/Em = 360/450 nm (Cutoff =420 nm, Blue).

EXAMPLE DATA ANALYSIS AND FIGURES

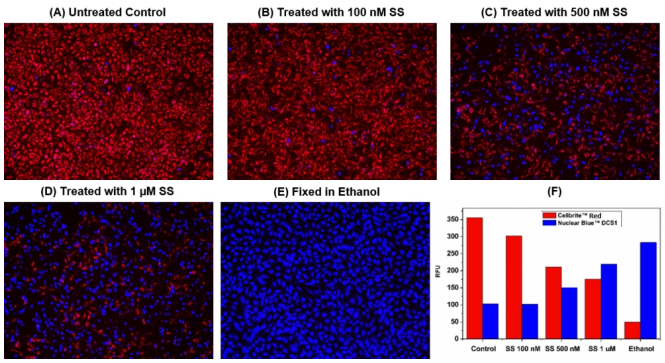

Figure 1. Fluorescence images of HeLa cells labeled with Live or Dead™ Cell Viability Assay Kit *Dual Fluorescence* (Cat#22788). HeLa cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were treated with 0-1 µM staurosporine (SS) at 37°C for 4 hours (A-D), or fixed in ethanol (E), then incubated with dye-loading solution for 1 hour. The

fluorescence signal was measured using a fluorescence microscope with Texas Red or Cy5 filter for viable cells (Red) and DAPI filter for necrotic cells (Blue), respectively. (F) The corresponding fluorescence signal were measured using a FlexStation® microplate reader (Molecular Devices) with bottom read mode at Ex/Em= 610/650 (cutoff=630 nm, Red) and Ex/Em=360/450 (cutoff=420 nm, Blue), respectively.

DISCLAIMER

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