Cell Meter™ Multiplexing Live, Apoptotic and Necrotic Cell Detection Kit III *Triple Fluorescence Colors*

Catalog number: 22846
Unit size: 200 Tests

Component	Storage	Amount
Component A: 100X Annexin V-iFluor™ 488 conjugate	Freeze (<-15 °C), Minimize light exposure	1 vial (200 uL)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (50 mL)
Component C: 200X Nuclear Blue™ DCS1	Freeze (<-15 °C), Minimize light exposure	1 vial (100 uL)
Component D: 200X Cellbrite™ Red	Freeze (<-15 °C), Minimize light exposure	1 vial (100 uL)

OVERVIEW

Our Cell Meter™ assay kits are a set of tools for monitoring cell viability. There are a variety of parameters that can be used. This particular kit is designed to simultaneously monitor apoptotic, necrotic and healthy cells. Apoptosis is an active, programmed process of autonomous cellular dismantling that avoids eliciting inflammation. In apoptosis, phosphatidylserine (PS) is transferred to the outer leaflet of the plasma membrane. As a universal indicator of the initial/intermediate stages of cell apoptosis, the appearance of phosphatidylserine on the cell surface can be detected before morphological changes are observed. The PS sensor Annexin V-iFluor™ 488 conjugate has green fluorescence upon binding to membrane PS. Necrosis has been characterized as passive, accidental cell death resulting from environmental perturbations with uncontrolled release of inflammatory cellular contents. Loss of plasma membrane integrity, as demonstrated by the ability of a membrane-impermeable Nuclear Blue™ DCS1 (Ex/Em = 350/461 nm) to label the nucleus, represents a straightforward approach to demonstrate late stage of apoptosis and necrosis. In addition, this kit also provides a live cell labeling dye, Cellbrite™ Red (Ex/Em = 613/631 nm), for labeling non-apoptotic healthy cells. This kit is optimized to simultaneously detect cell apoptosis (green), necrosis (blue and/or green) and healthy cells (red) with a fluorescence microscope.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds
2. Add triple fluorescence working solution (100 µL/sample)
3. Incubate at room temperature or 37°C for 30 - 60 minutes
4. Analyze with a fluorescence microscope at FITC channel (apoptosis), DAPI channel (necrosis) or Texas Red/Cy5 channel (healthy cells)

Important We treated HeLa cells with staurosporine (SS) for 4 hours at 37°C to induce cell apoptosis. See Figure 1 for details.
Thaw all components to room temperature before beginning protocol.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	494 nm (apoptosis) / 350 nm (necrosis) / 613 nm (live)
Emission:	520 nm (apoptosis) / 461 nm (necrosis) / 631 nm (live)
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	FITC filter (apoptosis) / DAPI filter (necrosis) / Cy5 filter (live)

PREPARATION OF WORKING SOLUTION

Add 10 µL of 100X Annexin V-iFluor™ 488 conjugate (Component A), 5 µL of 200X Nuclear Blue™ DCS1 (Component C) and 5 µL of 200X Cellbrite™ Red (Component D) to 1 mL of Assay Buffer (Component B). The triple fluorescence assay 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, C and D individually.

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

1. Remove cell culture medium and test compounds after treatment.
2. Add 100 µL/well (96-well plate) or 50 µL/well (384-well plate) of triple fluorescence assay solution. Incubate at 37°C for 30 to 60 minutes, protected from light.
3. Wash cells with HBSS, PBS or buffer of your choice twice.
4. Analyze the apoptotic cells with Annexin V-iFluor™ 488 conjugate under fluorescence microscope with a FITC filter. The green staining (Ex/Em = 494/520 nm) on the plasma membrane indicates the Annexin V-iFluor™ 488 conjugate binding to PS on cell surface. Monitor the fluorescence intensity with a DAPI filter (Ex/Em = 350/461 nm) for necrosis, Texas Red or Cy5 filter (Ex/Em = 613/631 nm) for live cells using a fluorescence microscope (See Figure 1 for details).

EXAMPLE DATA ANALYSIS AND FIGURES

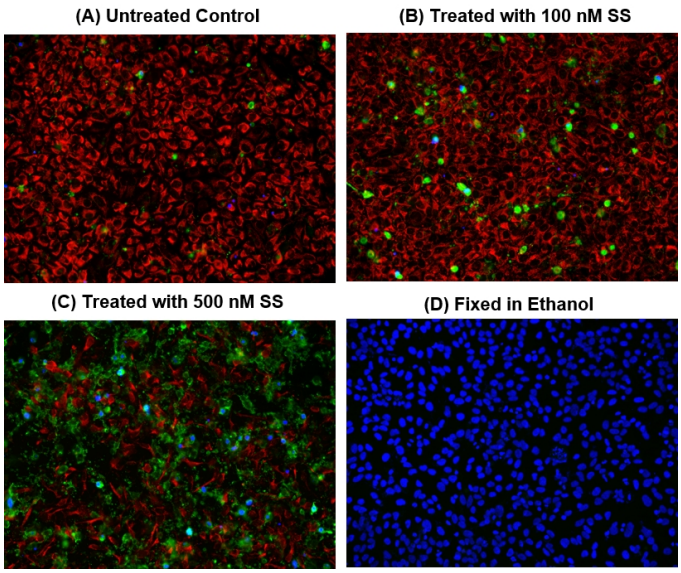

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Figure 1. Fluorescence images of HeLa cells labeled with Cell Meter™ Multiplexing Live, Apoptotic and Necrotic Detection Kit *Triple Fluorescence* (Cat#22846). HeLa cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were treated with 0-500 nM staurosporine (SS) at 37^{o}C for 4 hours (A-C), or fixed in ethanol (D), then incubated with triple fluorescence assay solution for 1 hour. The fluorescence signal was measured using a fluorescence microscope with a Cy5 filter for healthy cells (Red), FITC filter for apoptotic (Green) and DAPI filter for necrotic cells (Blue), respectively.

DISCLAIMER