Cell Meter™ Multiplexing Live, Apoptotic and Necrotic Cell Detection Kit III

**Triple Fluorescence **

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22846 (200 assays)	Keep at -20°C and avoid exposure to light	Fluorescence microscope

Introduction

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 (Ex/Em = 494/520 nm) 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.

Kit Components

Components	Amount
Component A: 100X Annexin V-iFluor™ 488 conjugate	1 vial (200 µL)
Component B: Assay Buffer	1 bottle (50 mL)
Component C: 200X Nuclear Blue™ DCS1	1 vial (100 µL)
Component D: 200X Cellbrite™ Red	1 vial (100 µL)

Assay Protocol

Brief Summary

Prepare cells with test compounds → Add triple fluorescence assay solution (200 µL/sample) → Incubate at room temperature or 37ºC for 30-60 minutes → Analyze with a fluorescence microscope at FITC channel (apoptosis), DAPI channel (necrosis) or Texas Red or Cy5 channel (healthy cells)

1. **Prepare cells:**
   Plate 100 to 100, 000 cells/well in a tissue culture microplate with black wall and clear bottom. Add test compounds into the cells and incubate for a desired period of time (such as 24, 48 or 96 hours) in a 37 °C, 5% CO_2 incubator. For blank wells (medium without the cells), add the same amount of compound buffer. The suggested total volume is 100 µL/well/96-well plate and 25 µL/well/384-well plate.

   Note: We treated HeLa cells with staurosporine (SS) for 4 hours at 37ºC to induce cell apoptosis. See Figure 1 for details.

2. **Prepare triple fluorescence assay solution:**

2.1 Prepare triple fluorescence assay solution by adding 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.

2.2 Remove cell culture medium and test compounds after treatment (from Step 1).

2.3 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of triple fluorescence assay solution. Incubate at room temperature or 37ºC for 30 to 60 minutes, protected from light.

2.4 Wash cells with HBSS, PBS or buffer of your choice twice.

2.5 Analyze the apoptotic cells with Annexin V-iFluor™ 488 under fluorescence microscope with a FITC filter. The green staining 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 for necrosis, Texas Red or Cy5 filter for live cells using a fluorescence microscope (See Figure 1 for details).

Data Analysis

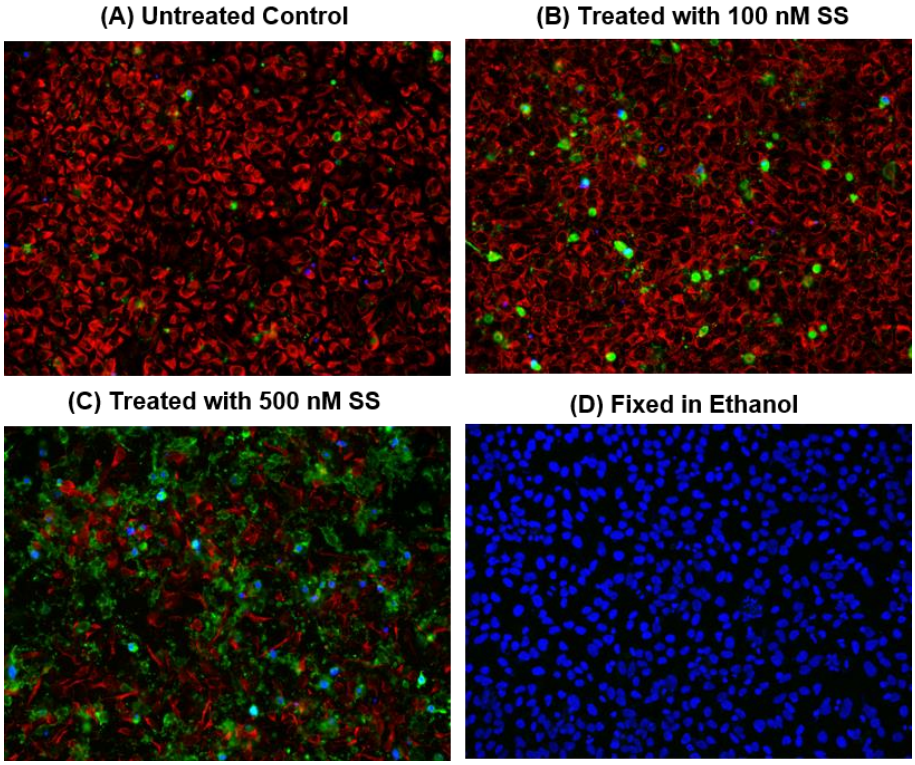

Figure 1. Fluorescence images of HeLa cells labeled with Cell Meter™ Multiplexing Live, Apoptotic and Necrotic Detection Kit *Triple Fluorescence* (Cat#22846). HeLa cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were treated with 0-500 nM staurosporine (SS) at 37 ºC 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.

References

1. Van Engeland M, Ramaekers FCS, Schutte B, Reutelingsperger CPM: A novel assay to measure loss of plasma membrane asymmetry during apoptosis of adherant cells in culture. Cytometry 24:131–139, 1996.
2. L Casciola-Rosen, A Rosen, M Petri, and M Schlissel. Surface blebs on apoptotic cells are sites of enhanced procoagulant activity: implications for coagulation events and antigenic spread in systemic lupus erythematosus. Proc Natl Acad Sci U S A. 1996 February 20; 93(4): 1624–1629.
3. Hanshaw RG, Lakshmi C, Lambert TN, Johnson JR, Smith BD. (2005) Fluorescent detection of apoptotic cells by using zinc coordination complexes with a selective affinity for membrane surfaces enriched with phosphatidylserine. Chembiochem, 6, 2214.