Cell Meter™ Apoptotic and Necrotic Detection Kit

Triple Fluorescence Color

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22843 (100 assays)	Keep at -20 °C and avoid exposure to light	Flow cytometer 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 used in this kit has red fluorescence (Ex/Em = 630/660 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 DNA Nuclear Green™ DCS1 (Ex/Em = 490/525 nm) to label the nucleus, represents a straightforward approach to demonstrate late stage apoptosis and necrosis. In addition, this kit also provides a live cell cytoplasm labeling dye CytoCalcein™ Violet 450 (Ex/Em = 405/450 nm) for labeling live cell cytoplasm. This kit is optimized to simultaneously detect cell apoptosis (Red), necrosis (green and/or red) and healthy cells (blue) with a flow cytometer or fluorescence microscope.

Kit Components

Components	**Amount**
Component A: 100X Apopxin™ Deep Red	1 vial (200 µL)
Component B: Assay Buffer	50 mL
Component C: 200X Nuclear Green™ DCS1	1 vial (100 µL)
Component D: CytoCalcein™ Violet 450	1 vial (lyophilized powder)

Assay Protocol

Brief Summary

Prepare cells with test compounds (200 µL/sample) → Add Apopxin™ Deep Red assay solution → Incubate at room temperature for 30-60 minutes → Analyze with a flow cytometer or a fluorescence microscope at Ex/Em = 630/660 nm (apoptosis), 503/526 nm (necrosis) and 405/450 nm (healthy cells)

1. Prepare and incubate cells with Apopxin™ Deep Red:

1.1 Treat cells with test compounds for a desired period of time (4-6 hours for Jurkat cells treated with staurosporine) to induce apoptosis.

1.2 Centrifuge the cells to get 1-5×10^5 cells/tube.

1.3 Resuspend cells in 200 µL of Assay Buffer (Component B).

1.4 Add 2 µL of Apopxin™ Red (Component A) into the cells.
Optional 1: Add 1 µL of 200X Nuclear Green™ DCS1 (Component C) into the cells for necrosis cells.
Optional 2: Add 100 µL of DMSO into the vial of CytoCalcein™ Violet 450 (Component D) to have 200X CytoCalcein™ Violet 450 stock solution, and then add 1 µL into the cells for healthy cells staining.

1.5 Incubate at room temperature for 30 to 60 minutes (protected from light).

1.6 Add 300 µL of Assay Buffer (Component B) to increase volume before analyzing the cells with a flow cytometer or fluorescence microscope (see Step 1.7 below).

1.7 Monitor the fluorescence intensity at Ex/Em = 630/660 nm for apoptosis**,** 490/520 nm for necrosis, and 405/450 nm for healthy cells using a flow cytometer or a fluorescence microscope (See Step 2 or 3 below).

2. Analyze cells using a flow cytometer:

Quantify Apopxin™ Red binding by using the FL4 channel (Ex/Em = 630/660 nm), and measure the cell viability using the FL1 channel (Ex/Em = 490/520 nm) when Nuclear Green™ DCS1 is added, and/or using Ex/Em = 405/450 nm when CytoCalcein™ Violet 450 is added into the cells.

Note: The flow cytometric analysis of Apopxin™ binding to adherent cells is not routinely tested since specific membrane damage may occur during cell detachment or harvesting. However, methods for utilizing Annexin V for flow cytometry on adherent cell types have been previously reported by Casiola-Rosen et al. and van Engelend et al (see Refs 1 and 2).

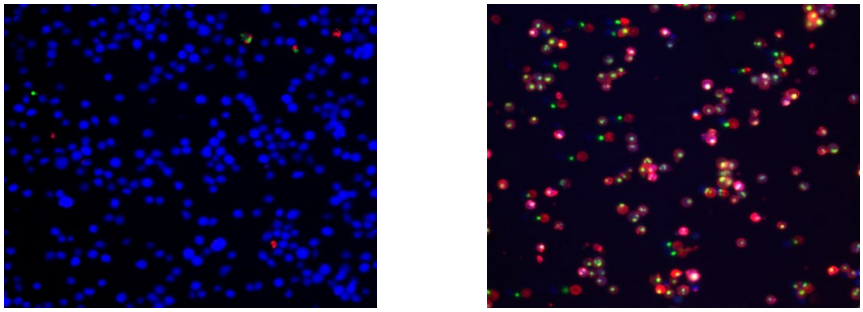
3. Analyze cells using a fluorescence microscope:

3.1 Pipette the cell suspension from Step 1.5, rinse 1-2 times with assay buffer, and then resuspend the cells with assay buffer. Add the cells on a glass slide that is covered with a glass cover slip or a black wall/clear bottom 96-well microplate.
Note: For adherent cells, it is recommended to grow the cells directly on a cover slip (or a black wall/clear bottom 96-well microplate). After incubation with Apopxin™ Deep Red (Step 1.5), rinse 1-2 times with assay buffer, and then add assay buffer back to the cover slip (or a black wall/clear bottom 96-well microplate). Invert cover slip on a glass slide and visualize the cells. The cells can also be fixed in 2% formaldehyde after the incubation with Apopxin™ Deep Red and visualized under a microscope.

3.2 Analyze the apoptotic cells with Apopxin™ Deep Red under a fluorescence microscope using the Cy5 channel. Measure the cell viability using the FITC channel when Nuclear Green™ DCS1 is added, and/or Violet channel when CytoCalcein™ Violet 450 is added into the cells. The red staining on the plasma membrane indicates the Apopxin™ Deep Red binding to PS on cell surface.

Data Analysis

In live non-apoptotic cells, Apopxin™ Deep Red detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells Apopxin™ Deep Red binds to phosphatidylserine, which is located on the outer leaflet of the cell membrane, resulted in increased staining intensity.



A. Live cells **B**. Apoptotic Cells

Figure 1. The detection of binding activity of Apopxin™ Deep Red to phosphatidylserine in Jurkat cells. The fluorescence image showing cells that are live (blue, stained by CytoCalcein™ Violet 450), apoptotic (red, stained by Apopxin™ Deep Red), and necrotic (green, indicated by Nuclear Green™ DCS1staining) in Jurkat cells induced by 1μM staurosporine for 3 hours. The fluorescence images of the cells were taken with Olympus fluorescence microscope through the Violet, Cy5 and FITC channel respectively. Individual images taken from each channel from the same cell population were merged as shown above. **A**: Non-induced control cells; **B**: Triple staining of staurosporine-induced cells.

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.