Cell Meter™ Apoptotic and Necrotic Multiplexing Detection Kit II *Triple Fluorescence Colors*

Catalog number: 22843
Unit size: 100 Tests

Component	Storage	Amount
Component A: 100X Apopxin™ Deep Red	Freeze (<-15 °C), Minimize light exposure	1 vial (200 µL)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (50 mL)
Component C: 200X Nuclear Green™ DCS1	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL)
Component D: CytoCalcein™ Violet 450	Freeze (<-15 °C), Minimize light exposure	1 vial (lyophilized powder)

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 described as 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 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 living 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.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds (200 µL/sample)
2. Add Apopxin™ Deep Red assay solution
3. Incubate at room temperature for 30 - 60 minutes
4. Analyze cells with a flow cytometer with 660/20 nm (for apotosis-APC channel), 530/30 nm (for necrosis-FITC channel) and 450/40 nm emission filter (for healthy cells-Pacific Blue channel) or fluorescence microscope with DAPI (Healthy cells), FITC (Necrosis cells) and Cy5 filters (Apoptotic cells)

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

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	405 nm, 488 nm, 633 nm laser
Emission:	450/40 nm, 530/30 nm, 660/20 nm filter
Instrument specification(s):	Pacific Blue, FITC, APC channel
Instrument:	Fluorescence microscope
Excitation:	DAPI, FITC, Cy5 filter sets
Emission:	DAPI, FITC, Cy5 filter sets
Recommended plate:	Black wall/clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. CytoCalcein™ Violet 450 stock solution (200X):
Add 100 µL of DMSO into the vial of CytoCalcein™ Violet 450 (Component D) to make 200X CytoCalcein™ Violet 450 stock solution. Protect from light.

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

Prepare and incubate cells with Apopxin™ Deep Red:

1. Treat cells with test compounds for a desired period of time (4-6 hours for Jurkat cells treated with staurosporine) to induce apoptosis.
2. Centrifuge the cells to get 1-5×10^5 cells/tube.
3. Resuspend cells in 200 µL of Assay Buffer (Component B).
4. Add 2 µL of 100X Apopxin™ Deep Red (Component A) into the cells.
5. **Optional 1:** Add 1 µL of 200X Nuclear Green™ DCS1 (Component C) into the cells for necrosis cells.
6. **Optional 2:** Then add 1 µL of 200X CytoCalcein™ Violet 450 stock solution into the cells for healthy cells staining.
7. Incubate at room temperature for 30 to 60 minutes, protected from light.
8. Add 300 µL of Assay Buffer (Component B) to increase volume before analyzing the cells with a flow cytometer or fluorescence microscope.
9. Monitor the fluorescence intensity using a flow cytometer with 660/20 nm (for apotosis-APC channel), 530/30 nm (for necrosis-FITC channel) and 450/40 nm emission filter (for healthy cells-Pacific Blue channel) or fluorescence microscope with DAPI (Healthy cells), FITC (Necrosis cells) and Cy5 filters (Apoptotic cells).

Analyze cells using a flow cytometer:

1. Quantify Apopxin™ Deep Red binding using a flow cytometer with 660/20 nm (for apotosis-APC channel), 530/30 nm (for necrosis-FITC channel) and 450/40 nm emission filter (for healthy cells-Pacific Blue channel).

   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.

Analyze cells using a fluorescence microscope:

1. Pipette the cell suspension after incubation, rinse 1-2 times with Assay Buffer,

and then resuspend the cells with Assay Buffer.

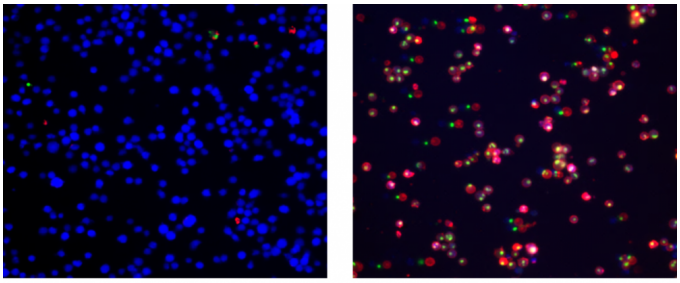
2. Add the cells on a glass slide that is covered with a glass cover-slip or a black wall/clear bottom 96-well microplate.

   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, 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. Analyze the apoptotic cells with Apopxin™ Deep Red under a fluorescence microscope using Cy5 filter. Measure the cell viability using FITC filter when Nuclear Green™ DCS1 is added, and/or Violet filter 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.

EXAMPLE DATA ANALYSIS AND FIGURES

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 using Cell Meter™ Apoptotic and Necrotic Mulptiplexing Detection Kit II. The fluorescence images 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™ DCS1 staining) in Jurkat cells induced by 1 μM 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.

DISCLAIMER