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

Catalog number: 22840
Unit size: 100 Tests

Component	Storage	Amount
Component A: 100X Apopxin™ Green	Freeze (<-15 °C), Minimize light exposure	1 vial (200 µL)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (50 mL)
Component C: 200X 7-AAD	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 monitor cell 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 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 7-AAD (Ex/Em = 546/647 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 detect cell apoptosis (green), necrosis (green and/or red) and healthy cells (blue) with a flow cytometer and fluorescence microscope.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds (200 µL/sample)
2. Add Apopxin™ Green assay solution
3. Incubate at room temperature for 30 - 60 minutes
4. Analyze cells with a flow cytometer with emission filter 530/30 nm (FITC channel- for apoptotic cells), 450/40 nm (Pacific Blue channel- for healthy cells) and 670/14 nm (PE-Cy5 channel- for necrotic cells)

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

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	405 nm, 488 nm laser
Emission:	450/40 nm, 530/30 nm, 670/14 nm filter
Instrument specification(s):	Pacific Blue, FITC, PE-Cy5 channel
Instrument:	Fluorescence microscope
Excitation:	DAPI, FITC, Texas Red filter
Emission:	DAPI, FITC, Texas Red filter
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™ Green:

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™ Green (Component A) into the cells.
5. **Optional 1:** Add 1 µL of 200X 7-AAD (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 emission filter 530/30 nm (FITC channel- for apoptotic cells), 450/40 nm (Pacific Blue channel- for healthy cells) and 670/14 nm (PE-Cy5 channel- for necrotic cells).

Analyze cells using a flow cytometer:

1. Quantify Apopxin™ Green binding using a flow cytometer with emission filter 530/30 nm (FITC channel- for apoptotic cells), 450/40 nm (Pacific Blue channel- for healthy cells) and 670/14 nm (PE-Cy5 channel- for necrotic 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.

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™ Green, 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™ Green and visualized under a microscope.

3. Analyze the apoptotic cells with Apopxin™ Green under a fluorescence microscope using the FITC filter. Measure the cell viability using Texas Red filter when 7-AAD is added, and/or DAPI or Violet filter when CytoCalcein™ Violet 450 is added into the cells. The green staining on the plasma membrane indicates the Apopxin™ Green binding to PS on cell surface.

EXAMPLE DATA ANALYSIS AND FIGURES

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

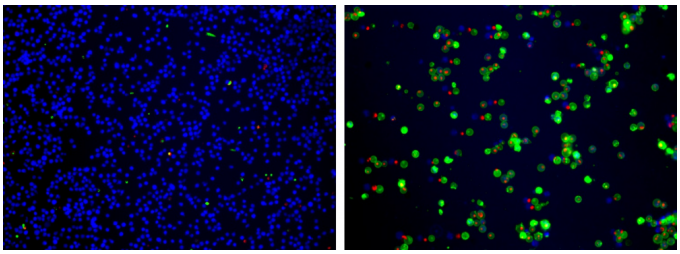

Figure 1. The fluorescence images showing cells that are live (blue, stained by CytoCalcein™ Violet 450), apoptotic (green, stained by Apopxin™ Green), and necrotic (red, indicated by 7-AAD staining) in Jurkat cells induced by 1µM staurosporine for 3 hours. The fluorescence images of the cells were taken with Olympus fluorescence microscope through the Violet, FITC and Texas Red channel respectively. Individual images taken from each channel from the same cell population were merged as shown above. Left: Non-induced control cells; Right: Triple staining of staurosporine-induced cells.

DISCLAIMER