Cell Meter™ Apoptotic and Necrotic Detection Kit

Tricolor Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22840 (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 green fluorescence (Ex/Em = 490/525 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 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 simultaneously detect cell apoptosis (green), necrosis (green and/or red) and healthy cells (blue) with a flow cytometer or fluorescence microscope.

Kit Components

Components	Amount
Component A: 100X Apopxin™ Green	1 vial (200 µL)
Component B: Assay Buffer	50 mL
Component C: 200X 7-AAD	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™ Green assay solution → Incubate at room temperature for 30-60 minutes → Analyze with a flow cytometer or a fluorescence microscope at Ex/Em = 490/525 nm (apoptosis), 550/650 nm (necrosis) and 405/450 nm (healthy cells)

1. Prepare and incubate cells with Apopxin™ Green:

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™ Green (Component A) into the cells.
Optional 1: Add 1 µL of 200X 7-AAD (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 = 490/525 nm for apoptosis**,** 550/650 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™ Green binding by using the FL1 channel (Ex/Em = 490/525 nm), and measure the cell viability using the FL3 channel (Ex/Em = 490/650 nm) when 7-AAD is added, and/or using Ex/Em = 405/450 nm when CytoCalcein™ Violet 450 is added into the cells.

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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™ Green (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™ Green and visualized under a microscope.

3.2 Analyze the apoptotic cells with Apopxin™ Green under a fluorescence microscope using the FITC channel. Measure the cell viability using the Texas Red channel when 7-AAD is added, and/or Violet channel 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.

Data Analysis

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.

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

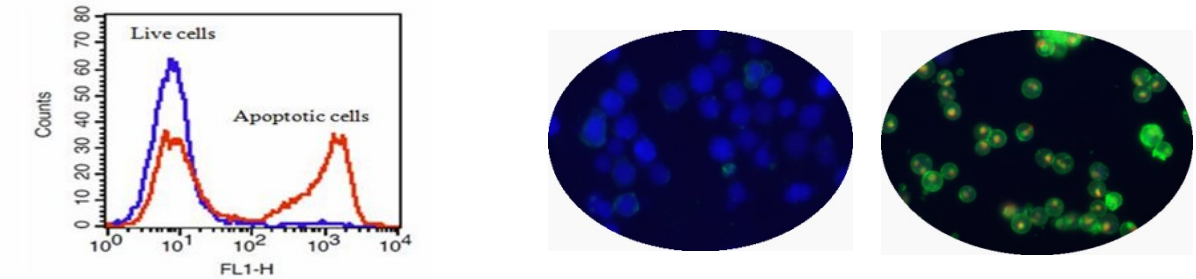

Figure 1. The detection of binding activity of Apopxin™ Green to phosphatidylserine in Jurkat cells. **A.** Jurkat cells were treated without (A. Blue) or with 1 µM staurosporine (A. Red) in a 37 °C, 5% CO_2 incubator for 5 hours, and then loaded with Apopxin™ Green for 30 minutes. The fluorescence intensity of Apopxin™ Green was measured with a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer using FL1 channel. **B and C:** The fluorescence image 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 TRITC channel respectively. Individual images taken from each channel from the same cell population were merged as shown above. **B**: Non-induced control cells; **C**: 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.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.