Cell Meter™ Phosphatidylserine Apoptosis Assay Kit

Blue Fluorescence Optimized for Microplate Readers

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22790 (100 assays)	Keep at 4 °C and protect from light	Fluorescence microscopes Fluorescence microplate readers

Introduction

Our Cell Meter™ assay kits are a set of tools for monitoring cellular functions. There are a variety of parameters that can be used to monitor cell viability. This particular kit is designed to monitor cell apoptosis by measuring the translocation of phosphatidylserine (PS). In apoptosis, PS is transferred to the outer leaflet of the plasma membrane. The appearance of phosphatidyserine on the cell surface is a universal indicator of the initial/intermediate stages of cell apoptosis and can be detected before morphological changes can be observed.

This kit uses our proprietary blue fluorescent Apopxin™ Violet 450 PS sensor that specifically binds PS with blue fluorescence. The stain has the spectral properties similar to those of Pacific Blue® at Ex/Em= ~405/450 nm (Pacific Blue® is the trademark of Invitrogen). The blue fluorescence stain is well excited with the violet laser at 405 nm, and emits intense blue fluorescence at ~450 nm. Due to its highly enhanced affinity to PS, this kit is more robust than other commercial Annexin V-based apoptosis kits that are only used with either microscope or flow cytometry platform. This kit is optimized for a fluorescence microplate reader besides the microscope platform.

Kit Components

Components	Amount
Component A: Apopxin™ Violet 450 (100X stock solution)	1 vial (100 µL)
Component B: Assay Buffer	10 mL

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare cells with test compounds (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Add equal volume of Apopxin™ Violet 450 assay solution → Incubate at room temperature for 1 hour → Monitor fluorescence intensity at Ex/Em = 405/450 nm (bottom read mode)

1. Prepare cells:

1.1 For adherent cells: Plate cells overnight in growth medium at 20,000 cells/well/90 µL for a 96-well plate or 5,000 cells/well/20 µL for a 384-well plate.

1.2 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellet in culture medium at 80,000 to 200,000 cells/well/90 µL for a 96-well poly-D lysine plate or 20,000 to 50,000 cells/well/20 µL for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

2. Prepare Violet 450 assay working solution:

2.1 Warm Kit Component B at room temperature before use.

2.2 Add 10 µL of Apopxin™ Violet 450 (Component A) into 1 mL of Assay Buffer (Component B), and mix them well.
Note: 100 µL of Apopxin™ Violet 450 assay working solution is enough for one well. Prepare fresh before use.

3. Run apoptosis assay:

3.1 Treat cells with test compounds by adding 10 µL/well (96-well plate) or 2.5 µL/well (384- well plate) of 10X test compound stock solution into PBS or the desired buffer. For blank wells (medium without the cells), add the same amount of compound buffer.

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3.2 Incubate the cell plate in a 5% CO_2, 37 °C incubator for a desired period of time (4-6 hours for Jurkat cells treated with staurosporine) to induce apoptosis.
Note: Some compounds such as camptothecin might give false positive response due to the broad emission spectrum from 380 to 490 nm when excited at 405 nm.

3.3 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of 2X Apopxin™ Violet 450 assay working solution (from Step 2.2) into each well.

3.4 Incubate the cell plate at room temperature for at least 1 hour, protected from light.

3.5 Centrifuge cell plate (especially for the non-adherent cells) at 800 rpm for 2 minutes (brake off).

3.6 Monitor the fluorescence intensity at Ex/Em = 405/450 nm (cut off at 420 nm) by using a fluorescent microplate reader (bottom read mode) or using a fluorescent microscope (FITC channel).

Data Analysis

The fluorescence in blank wells with the growth medium is subtracted from the values for those wells with the cells. The background fluorescence of the blank wells may vary depending on the sources of the growth media or the microtiter plates.

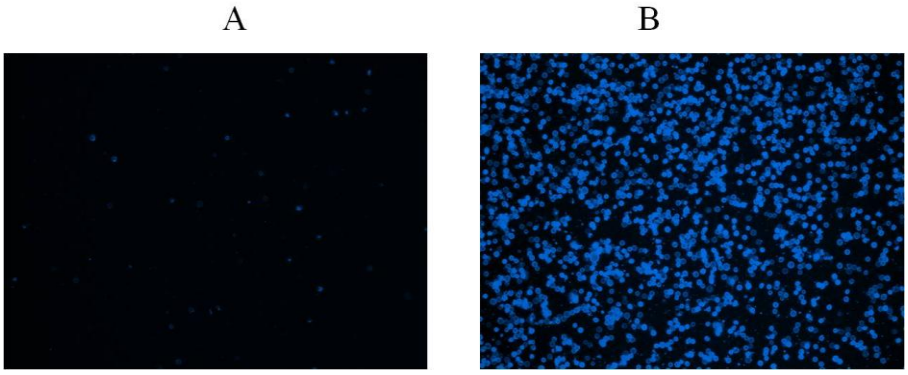

Figure 1. Detection of Apopxin™ Violet 450-PS binding activity in Jurkat cells. Jurkat cells were seeded on the same day at 200,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with or without 1 µM staurosporine for 5 hours. The Apopxin™ Violet 450 assay solution (100 µL/well) was added and incubated at room temperature for 1 hour. The fluorescence intensity was measured at Ex/Em = 405/450 nm with FlexStation (from Molecular Devices) using bottom read mode.

References

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