Cell Meter™ Phosphatidylserine Apoptosis Assay Kit

Deep Red Fluorescence, Optimized for Microplate Readers

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22793 (100 assays)	Keep at 4 °C and protect from light	Fluorescence microscope 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 phosphatidylserine 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 red fluorescent Apopxin™ PS sensor that specifically binds PS with affinity much higher than Annexin V (K_d < 10 nM). The PS sensor used in this kit has red fluorescence upon binding to membrane PS. The stain has the spectral properties almost identical to those of Cy5® or Alexa Fluor® 647, making it convenient to be used with the common fluorescence instruments equipped with the light sources and filters for Cy5® or Alexa Fluor® 647 (Cy5® or Alexa Fluor® 647 are the trademarks of GE Healthcare and Invitrogen respectively). Due to its highly enhanced affinity to PS, this kit is more robust than other commercial Annexin-V based apoptosis kits and can be used with a fluorescence microplate reader and microscope platforms.

Kit Key Features

Non-Radioactive:	No special requirements for waste treatment.
Continuous:	Easily adapted to automation with minimal hands on time.
Convenient:	All essential assay components are included.
Optimized Performance:	Optimized for detecting the translocation of phosphatidylserine (PS).
Enhanced value:	Less expensive than the sum of individual components.

Kit Components

Components	Amount
Component A: Apopxin™ Deep Red (100X Stock Solution)	1 vial (100 µL/vial)
Component B: Assay Buffer	10 mL

Materials and Instruments Required (but not provided)

- 96 or 384-well microplates: Tissue culture microplates with black wall and clear bottom are recommended.
- A fluorescence microplate reader: Capable of detecting excitation and emission at 650/680 nm with bottom read mode.
- A fluorescence microscope.

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™ Deep Red assay solution → Incubate at room temperature for 1 hour → Monitor fluorescence intensity at Ex/Em = 650/680 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

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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 Apopxin™ Deep Red assay solution:

2.1 Warm Kit Component B at room temperature before use.

2.2 Add 10 µL of Apopxin™ Deep Red (Component A) into 1 mL of Assay Buffer (Component B), and mix them well.
Note: 100 µL of Apopxin™ Deep Red assay 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.

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 camptothecin) to induce apoptosis.

3.3 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Apopxin™ Deep Red assay 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 = 650/680 nm (cut off at 660 nm) by using a fluorescent microplate reader (bottom read mode) or using a fluorescent microscope (Cy5® channel).

4. 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.

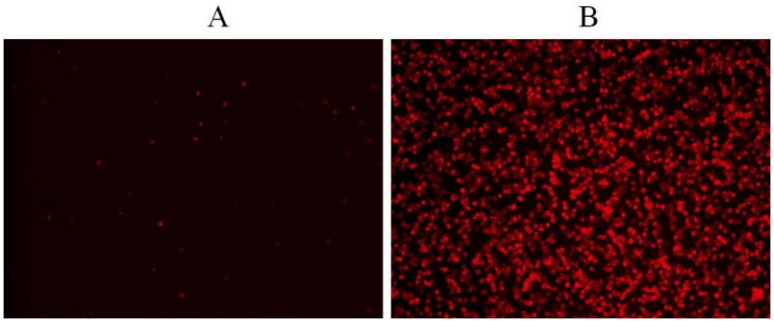

Figure 1. Images of Jurkat cells in a Costar black wall/clear bottom 96-well plate stained with the Cell Meter™ Phosphatidylserine Apoptosis Assay Kit. A: Untreated control cells. B: Cells treated with 20 µM camptothecin for 5 hours.

References

1. 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.
2. Koulov AV, Stucker KA, Lakshmi C, Robinson JP, Smith BD. (2003) Detection of apoptotic cells using a synthetic fluorescent sensor for membrane surfaces that contain phosphatidylserine. Cell Death Differ, 10, 1357.
3. Hall MP, Burson KK, Huestis WH. (1998) Interactions of a vesicular stomatitis virus G protein fragment with phosphatidylserine: NMR and fluorescence studies. Biochim Biophys Acta, 1415, 101.