

ab324124 – Phosphatidylserine Apoptosis Assay Kit (Fluorometric)

A set of tools for monitoring cell viability.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab324124

Storage and Stability: Store kit at +4°C in the dark immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components.

Materials Supplied

Item	Quantity	Storage Condition
Apopxin™ Green (100X Stock Solution)	1 vial (100 µL/vial)	+4°C (Store in the dark)
Assay Buffer	10 mL	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Fluorescence microplate reader (490/525 nm) – Bottom read mode
- Fluorescence microscope – FITC filter
- 37°C incubator
- Black wall/ clear bottom plates

Protocol Summary

1. Prepare cells with test compounds (100 µL/well/96-well plate or 25 µL/well/384-well plate).
2. Add equal volume of Apopxin™ Green working solution.
3. Incubate at room temperature for 1 hour.
4. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 490/525 nm (Cutoff = 515 nm) or fluorescence microscope with FITC filter.

IMPORTANT: Warm Assay Buffer at room temperature before starting the experiment.

Preparation of Working Solution

Apopxin™ Green working solution

1. Add 10 µL of 100X Apopxin™ Green into 1 mL of Assay Buffer and mix well to make Apopxin™ Green working solution.

Note: 100 µL of Apopxin™ Green working solution is enough for one well. Prepare fresh before use.

Experimental Protocol

1. Treat cells with desired test compounds by adding 10 µL/well (96-well plate) of 10X test compound solution into cell preparation buffer, for a total volume of 100 µL/well. For a 384-well plate, use 5 µL/well of 5X test compound solution into cell preparation buffer, for a total volume of 25 µL/well. For blank wells (medium without the cells), add the same amount of compound buffer.
2. Incubate the cell plate in a 5% CO₂, 37°C incubator for a desired period of time (4 - 6 hours for Jurkat cells treated with Camptothecin) to induce apoptosis.
3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Apopxin™ Green working solution into each well.
4. Incubate the cell plate at room temperature for at least 1 hour, protected from light.
5. Centrifuge cell plate (especially for the non-adherent cells) at 800 rpm for 2 minutes (brake off).
6. Monitor the fluorescence intensity with a fluorescence microplate reader (bottom read mode) at Ex/Em = 490/525 nm (Cutoff = 515 nm) or image cells using fluorescence microscope with FITC filter.

Data Analysis

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Camptothecin samples.

For technical support contact information, visit: www.abcam.com/contactus

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