ab176749 Apoptosis/Necrosis Assay Kit (blue, green, red)

For detection of apoptosis and necrosis in adherent or suspension cells.
This product is for research use only and is not intended for diagnostic use.

Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Grow cells
- Treat cells with appropriate agents and incubate for desired period of time
- Stain cells with Apopxin Green Solution, 7-ADD and CytoCalcein Violet 450
- Incubate cells at RT 30 – 60 minutes protected from light
- OPTIONAL: treat cells with cytotoxic agent and incubate for desired period of time
- Analyze results in a flow cytometer or fluorescence microscope at
  Ex/Em = 490/525 nm (apoptosis)
  Ex/Em = 550/650 nm (necrosis)
  Ex/Em = 405/450 nm (healthy cells)

Precautions

- read these instructions carefully prior to beginning the assay.
- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 6 months from receipt, providing components have not been reconstituted.
- Refer to list of materials supplied for storage conditions of individual components, both before and after preparation.
- Aliquot components in working volumes before storing at the recommended temperature. Reconstituted components are stable for 3 months.

Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Apopxin Green Indicator	200 µL	-20°C (In Dark)	-20°C (In Dark)
Assay Buffer	50 mL	-20°C	-20°C
200x 7-AAD	100 µL	-20°C (In Dark)	-20°C (In Dark)
CytoCalcein Violet 450	1 vial	-20°C (In Dark)	-20°C (In Dark)

Materials Required, Not Supplied

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

- Fluorescence microplate reader or flow cytometer.
- MilliQ water or other type of double distilled water (ddH2O)
- Pipettes and pipette tips, including multichannel pipette
- General tissue culture supplies
- PBS
- DMSO (anhydrous, cell culture grade)
- Sterile, tissue culture treated, clear bottom, dark sided 96-well microplates
- (Optional) Coverslips – if growing cells in 12-well/24-well culture plates for immunofluorescence detection
- (Optional) Cell scraper – for harvesting adherent cells for flow cytometry detection
- (Optional) 2% formaldehyde (v/v) in ddH2O – for cell fixation

Reagent Preparation

- Thaw all the kit components at room temperature before starting the experiment.
- Briefly centrifuge small vials at low speed prior to opening.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use.
Apopxin Green Indicator (100X): Ready to use as supplied. Protect from light.
7-AAD 200X: Ready to use as supplied. Protect from light.
CytoCalcein Violet 450: Add 100 µL of DMSO (not provided) into the vial to prepare a 200X stock solution. Resuspend thoroughly by pipetting up and down. Aliquot dye so that you have enough volume to perform the desired number of assays. Avoid freeze/thaw cycles. Store at -20°C in the dark. Reconstituted CytoCalcein is stable for 3 months. Keep on ice while in use.

Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to.
- Assay all controls and samples in duplicate.
- The reagents (Apopxin, 7-AAD and CytoCalcein) present in this kit are light sensitive. Maintain reagents and labeled cells in the dark.

1.1 Grow and treat cells of interest with appropriate compounds to induce apoptosis.

Treatment times may vary depending on the agent and cell line. Suggested positive control: Jurkat cells treated with 1 µM staurosporine for 4 – 6 hours will show clear apoptotic signal.

1.2 Flow Cytometry assay:

1. Collect untreated/treated cells so that you have 1 – 5x105 cells/tube.

Suspension cells: transfer cells to collection tube directly.
Adherent cells: adherent cells can be collected by carefully scrapping sample in cold PBS. However, this membrane damage may occur during cell detachment or harvesting which can lead to an increase in background signal.

2. Centrifuge cells at 500 x g for 5 minutes in a cold centrifuge. Discard supernatant.
3. Resuspend cells in 200 µL of Assay Buffer.
4. Add 2 µL of Apopxin Green Indicator (100X) to cells.
5. Add 1 µL of 7-AAD 200X to cells if detecting necrotic cells.
6. Add 1 µL CytoCalcein 450 200X Stock solution to cells if detecting healthy cells.

Δ Note: Alternatively, for an easier procedure, staining solutions can be added to Assay Buffer to create a staining mix before cell resuspension. In that case, add 204 µL of Assay Buffer/ Apopxin/ 7-AAD/CytoCalcein mix to each tube of cells.

7. Incubate cells at room temperature for 30 – 60 minutes.
8. Add 300 µL of Assay Buffer to increase volume before analyzing cells with a flow cytometer.
9. Quantify Apopxin Green Indicator binding by using the FL1 channel (Ex/Em = 490/525 nm), measure cell viability with 7-AAD by using the FL3 channel (Ex/Em = 550/650 nm) and/or using the Violet channel (Ex/Em = 405/450 nm) when CytoCalcein Violet 450 is added to the cells.

1.3 Fluorescence Microscopy assay:

Δ Note: Procedure described in this section has been optimized for 96-well microplates. Volumes can be scaled up to adapt protocol for larger culture plates.

1. Grow 1 – 5x105 cells/well in a 96-well microplate (black wells/clear flat bottom).

Suspension cells: cells can be attached to the bottom of plates by centrifuging plates in an appropriate plate-adapted centrifuge.

Adherent cells: number of cells depend on the cell type (general recommendation below).

CHO-K1 cells: 5 – 8x104 cells/well.

HeLa cells: 3 – 5x104 cells/well.

Δ Note: cells can also be grown on coverslips in 12-well/24-well culture plates. Volumes should be adjusted accordingly to ensure cells are covered at all times

2. Wash cells 1 – 2 times with 100 µL Assay Buffer, by carefully pipetting buffer up and down.
3. Resuspend cells in 200 µL of Assay Buffer.Add 2 µL of Apopxin Green Indicator (100X) to cells.
4. Add 1 µL of 7-AAD 200X to cells if detecting necrotic cells.

Δ Note: Cells can be fixed in 2% formaldehyde after Apopxin/7-AAD staining. If cells have been fixed, skip next step and go to step 7.

5. Add 1 µL CytoCalcein 450 200X Stock solution to cells if detecting healthy cells. NOTE: CytoCalcein dye cannot be fixed or used on fixed cells.

Δ Note: alternatively, for an easier procedure, staining solutions can be added to Assay Buffer to create a staining mix before cells resuspension. In that case, add 204 µL of Assay Buffer/ Apopxin/ 7-AAD/ CytoCalcein mix to each tube of cells.

6. Incubate cells at room temperature for 30 – 60 minutes.
7. Wash cells 1 – 2 times with 100 – 200 µL Assay Buffer. Replace with 100 – 200 µL Assay Buffer.
8. Analyze cells under the fluorescence microscope: apoptotic cells will show green staining due to the binding of Apopxin Green Indicator to PS and can be visualized using the FITC channel (Ex/Em = 490/525 nm). Analyze cell viability using the Texas Red channel (Ex/Em = 550/650 nm) after 7-AAD staining and/or violet channel (Ex/Em = 405/450 nm) after addition of CytoCalcein Violet 450.

Data Analysis

In live non-apoptotic cells, Apopxin Green detects innate apoptosis in non-treated cells, which is typically 2 – 6% of all cells. The table below can be used as guidance for interpretation of results:

	Channel/ color	Apoptotic cell	Necrotic cell	Viable cell
Apopxin Green	Green	Yes	No	No
7-AAD	Red	Yes (at late stage)	Yes	No
Cytocalcein violet 450	Blue	No	No	Yes

Flow Cytometry Measurement:

Exclude debris and isolate cell population of interest with gating. Using mean fluorescent intensity, determine fold change between control and treated samples.

Fluorescence Microscopy Measurement:

Blind count (e.g., covering sample name to avoid subjective bias) a sufficient number of cells (suggestion >200 cells/staining) to ensure observations are representative of the sample. Determine change (% of cells stained with each dye) between control and treated samples.

FAQs

Can I use fixed my cells before or after staining?

If you want to observe the three populations (viable, apoptotic and necrotic cells), you can only use live cells. Both Apopxin Green and 7-AAD can be fixed after staining, but that is not the case for cytocalcein. Cytocalcein cannot be fixed as it has no group to link with proteins using formaldehyde during fixation and will leak out of the cell and wash off after fixation. Moreover, if the sample has been fixed after Apopxin/7-AAD staining, cytocalcein cannot be used.

What is the principal of this kit?

The Apopxin is used to monitor cell apoptosis through measuring the translocation of phosphatidylserine (PS). In apoptosis, PS is transferred to the outer leaflet of the plasma membrane. The appearance of PS 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. The Apopxin used in this kit is a small molecule-based PS sensor. It has green fluorescence upon binding to membrane PS.

My cells don't look healthy after using the assay buffer. Why is this?

Your cells may be sensitive to calcium. Our assay buffer contains 1.5 mM higher calcium than the normal buffer such as Hanks and Hepes buffer (HHBS,1.2 mM). You could therefore use either medium or HHBS for washing.

Technical Contacts

For all technical and commercial enquires please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

http://www.abcam.co.jp/contactus(Japan)