ab176750 Apoptosis/Necrosis Detection Kit (blue, red, green)

Instructions for use:

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.

Version 2c Last Updated 12 December 2018

Table of Contents

1. BACKGROUND

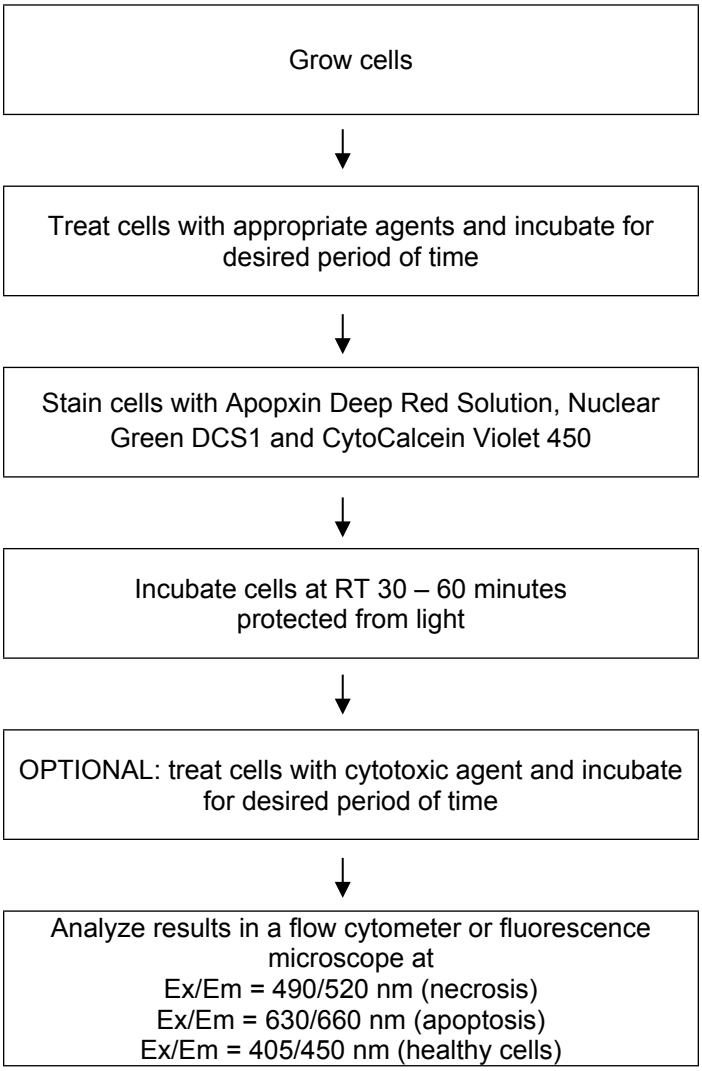
Abcam's Apoptosis/ Necrosis Detection Kit (blue, red, green) (ab176750) is designed to simultaneously monitor apoptotic, necrotic and healthy cells with a flow cytometer or fluorescence microscope.

- Phosphatidylserine (PS) exposure – a hallmark of apoptosis. Upon binding to the PS sensor, red fluorescence (Ex/Em = 630/660 nm) can be observed.
- Loss of plasma membrane integrity – a characteristic event in late apoptosis and necrosis. DNA Nuclear Green DCS1, a membrane-impermeable dye, will label the nucleus of damaged cells which can be observed as green fluorescence, (Ex/Em = 490/525 nm).
- Live cell staining – live cells can be easily differentiated with CytoCalcein Violet 450, a dye that is sequestered in the cytoplasm of live cells, and can be detected as blue fluorescence (Ex/Em = 405/450 nm).

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.

Necrosis is generally described as passive, accidental cell death resulting from environmental disturbances with uncontrolled release of inflammatory cellular contents.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please 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.

4. STORAGE AND STABILITY

Store kit at -20ºC 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. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 3 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Apopxin Deep Red Indicator	200 µL	-20°C	-20°C
Assay Buffer	50 mL	-20°C	-20°C
Nuclear Green DCS1	100 µL	-20°C	-20°C
CytoCalcein Violet 450	1 vial	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

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

- Fluorescence microplate reader or flow cytometer.
- MilliQ water or other type of double distilled water (ddH_2O)
- 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 ddH_2O – for cell fixation

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice. Clear bottom, dark sided microplates are recommended with this assay. Clear sided microplates have not been tested with this kit.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1. **Assay Buffer:**

Ready to use as supplied. Store at -20°C. Equilibrate to room temperature before use.

9.2. **Apopxin Deep Red Indicator (100X):**

Ready to use as supplied. Aliquot Indicator so that you have enough volume to perform the desired number of assays. Avoid freeze/thaw cycles. Store at -20°C in the dark. Keep on ice while in use.

9.3. **Nuclear Green DCS1:**

Ready to use as supplied. Aliquot Nuclear Green DCS1 so that you have enough volume to perform the desired number of assays. Avoid freeze/thaw cycles. Store at -20°C in the dark. Keep on ice while in use.

9.4. **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.

10. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to correct temperature prior to use.**
- **We recommended to assay all controls and samples in duplicate.**
- **The reagents (Apopxin, Nuclear Green and CytoCalcein) present in this kit are light sensitive. Maintain reagents and labeled cells in the dark.**

10.1. **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.

10.2. **Flow cytometry assay:**

10.2.1. Collect untreated/treated cells so that you have 1 – 5x10^5 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.

10.2.2. Centrifuge cells at 500 x *g* for 5 minutes in a cold centrifuge. Discard supernatant.

10.2.3. Resuspend cells in 200 µL of Assay Buffer.

10.2.4. Add 2 µL of Apopxin Deep Red Indicator (200X) to cells.

10.2.5. Add 1 µL of Nuclear Green 200X to cells if detecting necrotic cells.

10.2.6. Add 1 µL CytoCalcein 450 200X Stock solution to cells if detecting healthy cells.

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/ Nuclear Green/ CytoCalcein mix to each tube of cells.*

10.2.7. Incubate cells at room temperature for 30 – 60 minutes.

10.2.8. Add 300 µL of Assay Buffer to increase volume before analyzing cells with a flow cytometer.

10.2.9. Quantify Apopxin Deep Red Indicator binding by using the FL4 channel (Ex/Em = 630/660 nm), measure cell viability with Nuclear Green DCS1 by using the FL1 channel (Ex/Em = 490/520 nm) and/or using the Violet channel (Ex/Em = 405/450 nm) when CytoCalcein Violet 450 is added to the cells.

10.3. **Fluorescence microscopy assay:**

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.*

10.3.1. Grow 1 – 5x10^5 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 – 8x10^4 cells/well.

HeLa cells: 3 – 5x10^4 cells/well.

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.*

10.3.2. Wash cells 1 – 2 times with 100 µL Assay Buffer, by carefully pipetting buffer up and down.

10.3.3. Resuspend cells in 200 µL of Assay Buffer.

10.3.4. Add 2 µL of Apopxin Deep Red Indicator (100X) to cells.

10.3.5. Add 1 µL of Nuclear Green 200X to cells if detecting necrotic cells.

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 10.3.7*

10.3.6. 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.*

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/ Nuclear Green/ CytoCalcein mix to each tube of cells.*

10.3.7. Incubate cells at room temperature for 30 – 60 minutes.

10.3.8. Wash cells 1 – 2 times with 100 – 200 µL Assay Buffer. Replace with 100 – 200 µL Assay Buffer.

10.3.9. Analyze the apoptotic cells with Apopxin™ Deep Red under a fluorescence microscope using the Cy5 channel (Ex/Em = 630/660 nm). Measure the cell viability using the FITC channel when Nuclear Green™ DCS1 is added (Ex/Em = 490/520 nm), and/or Violet channel (Ex/Em = 405/450 nm) when CytoCalcein™ Violet 450 is added into the cells. The red staining on the plasma membrane indicates the Apopxin™ Deep Red binding to PS on cell surface.

11. DATA ANALYSIS

In live non-apoptotic cells, Apopxin Deep Red Indicator detects innate apoptosis in non-induced 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 Deep Red	Red	Yes	No	No
Nuclear Green DCS1	Green	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.

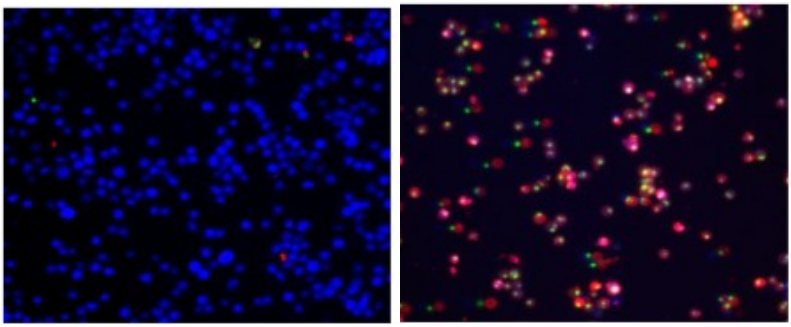


Figure 1: Fluorescence microscope analysis in Jurkat cells. Jurkat cells were either untreated (left image) or treated with 1 µM staurosporine (right image) in a 37°C, 5% CO_2 incubator for 3 hours. Images show cells that are alive (blue, stained with CytoCalcein Violet 450), apoptotic cells (red, stained with Apopxin Deep Red Indicator) and necrotic cells (green, stained with Nuclear Green DCS1). Image on the left shows mostly healthy cells with few dead cells scattered around, whereas image on the right shows mainly apoptotic cells with few necrotic cells. Images were taken with an Olympus fluorescence microscope through the violet, Cy5 and FITC channel respectively. Individual images taken from each channel from the same population were merged as shown above.

12. FAQs

Can I fix 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 Deep red and Nuclear Green 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.

13. NOTES

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940