Version 2b Last updated 10 March 2026

ab219916 Annexin V-iFluor 488 Apoptosis Detection Kit

For the rapid, sensitive and accurate measurement of PS exposure in live cells

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

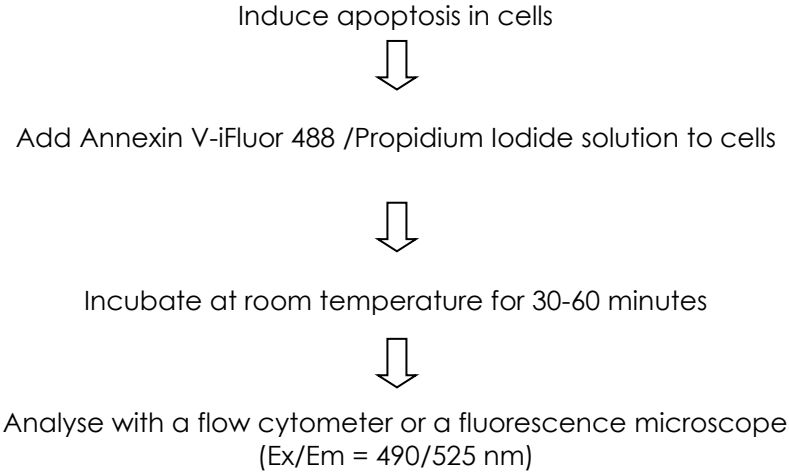
Annexin V-iFluor 488 Apoptosis Detection Kit (ab219916) contains Annexin V labeled with our proprietary green fluorescent dye iFluor 488, which allows the identification and quantitation of apoptotic cells on a single-cell basis by flow cytometry. Simultaneous staining of cells with Annexin V-iFluor488 (green fluorescence) and the non-vital dye propidium iodide (PI) (orange fluorescence) allows the discrimination of intact cells (Annexin V-iFluor 488 negative, PI Staining Solution negative), early apoptotic (Annexin V-iFluor 488 positive, PI Staining Solution negative) and late apoptotic or necrotic cells (Annexin V-iFluor 488, PI Staining Solution positive).

The iFluor 488 dye (Ex/Em = 490/525 nm) has spectral properties almost identical to those of FITC or Alexa Fluor® 488, making it convenient to be used with the common fluorescence instruments equipped with the light sources and filters for FITC, the most common fluorophore.

Apoptosis is a regulated process of cell death that occurs during embryonic development as well as maintenance of tissue homeostasis. Inappropriately regulated apoptosis is implicated in different disease states, such as neurodegeneration disease and cancer. The apoptosis program is characterized by morphologic features, including loss of plasma membrane asymmetry and attachment, condensation off the cytoplasm and nucleus, and compaction and fragmentation of the nuclear chromatin. Exposure of phosphatidylserine (PS) on the external surface of the cell membrane has been reported to occur in the early phases of apoptotic cell death, during which the cell membrane remains intact. In leukocyte apoptosis, PS on the outer surface of the cell marks the cell for recognition and phagocytosis by macrophages. The human vascular anticoagulant, annexin V, is a 35-36 kDa Ca^{2+} dependent phospholipid binding protein that has a high affinity for PS, and shows minimal binding to phosphatidylcholine and sphingomyelin. Changes in PS asymmetry, which can be analyzed by measuring annexin V binding to the cell membrane, are generally observed before morphological changes associated with apoptosis occurred and before membrane integrity is lost.

Alexa Fluor® 488 is the trademark of Invitrogen.

2. Protocol 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.
- 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.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website www.abcam.com.

4. Storage and Stability

Store kit at in the dark immediately upon receipt at temperatures shown below. Kit has a storage time of 1 year 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.

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	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Annexin V-iFluor 488 (100X stock solution)	200 µL	4°C	4°C
Assay Buffer	50 mL	4°C	4°C
100X Propidium Iodide	100 µL	-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:

- Flow cytometer or fluorescence microscopy with a filter capable of detecting fluorescence at Ex/Em = 490/525 nm
- Phosphate-buffered saline (PBS)
- Pipettes and pipette tips, including multi-channel pipette
- Tubes for the preparation of reagents and buffer solutions
- General tissue culture supplies

For flow cytometry assay:

- 12 x 75 mm tubes for flow cytometry

For fluorescence microscopy assay:

- Sterile 96-well plate with clear flat bottom, preferably black. Use a poly-D-lysine coated plate for suspension cells

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 and reagents by changing tips between sample and reagent additions.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- 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 Annexin V-iFluor 488 (100X stock solution):

Ready to use. Equilibrate to room temperature before use. Store at 4°C protected from light. Keep on ice while in use.

9.2 Assay Buffer:

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

9.3 100X Propidium Iodide:

Ready to use. Equilibrate to room temperature before use. Store at -20°C protected from light.

10. Assay Procedure – flow cytometry

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.

10.1 Prepare and incubate cells:

10.1.1 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 controls:

- Jurkat cells treated with 1 µM staurosporine for 5 hours
- Jurkat cells treated with 6 µM camptothecin for 4 hours

Δ Note: a negative control with untreated cells should be included in the analysis. The negative control is used to define the basal level of apoptotic and necrotic or dead cells.

10.1.2 Additional controls necessary to set up flow cytometer compensation and quadrants (first time use only):

- Untreated unstained cells
- Untreated cells stained with Annexin V-iFluor 488 only
- Untreated cells stained with PI alone

10.2 Harvest and stain cells:

10.2.1 Harvest untreated and treated cells (1-5 x 10^5 cells/tube).

- Suspension cells: transfer cells to collection tube directly.
- Adherent cells: gently trypsinize and wash cells once with serum-containing media. Be aware that membrane damage may occur during cell detachment or harvesting which can lead to an increase in background signal.

10.2.2 Wash cells twice at room temperature in PBS.
10.2.3 Resuspend cells in 200 µL of Assay Buffer.
10.2.4 Add 2 µL of Annexin V-iFluor 488 conjugate + 2 µL of 100X Propidium Iodide to cells for dead/necrotic cells.

Δ Note: alternatively, for an easier procedure, Annexin V conjugate and Propidium Iodide can be added to assay buffer before cells resuspension. In that case, add 204 µL of the staining mix to each tube of cells.

10.2.5 Incubate at room temperature for 30-60 minutes, protected from light.

10.2.6 Optional: add 200-300 µL of assay buffer to increase volume before analyzing the cells with a flow cytometer.

10.3 Analyze cells by flow cytometry:

Δ Note: we recommend analyzing cells within one hour of staining.

10.3.1 Set appropriate FSC vs SSC gates to exclude debris and cell aggregates.

10.3.2 Use set up controls (Step 10.1.2) to set up necessary laser compensations.

10.3.3 Monitor the fluorescence intensity of Annexin V-iFluor and propidium iodide in the appropriate channel (iFluor 488, Ex/Em = 490/525 nm FL1 channel; PI, FL2 channel).

11. Assay Procedure – fluorescence microscopy

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.
- The procedure described in this section has been optimized for 96-well microplates. Volumes can be scaled up to adapt protocol for larger culture plates.

11.1 Prepare and grow cells:

11.1.1 Suspension cells:

- Grow 1-5 x 10^5 cells/well in a 96-well microplate (black wells/clear flat bottom)
- Cells can be attached to the bottom of plates by centrifuging plates in an appropriate plate-adapted centrifuge.

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

- CHO-K1 cells: 5-8 x 10^4 cells/well
- HeLa cells: 3-5 x 10^4 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 always covered.

11.1.3 Treat cells with appropriate compounds to induce apoptosis. Treatment times may vary depending on the agent and cell line. Suggested positive controls:

- Jurkat cells treated with 1 µM staurosporine for 5 hours
- Jurkat cells treated with 6 µM camptothecin for 4 hours

Δ Note: A negative control with untreated cells should be included in the analysis. The negative control is used to define the basal level of apoptotic and necrotic or dead cells.

11.2 Stain cells:

11.2.1 Wash cells twice at room temperature in assay buffer.
11.2.2 Add 200 µL of assay buffer to cells.
11.2.3 Add 2 µL of Annexin V-iFluor 488 conjugate + 2 µL Propidium Iodide to cells for dead/necrotic cells.

Δ Note: alternatively, for an easier procedure, Annexin V conjugate and Propidium Iodide can be added to assay buffer before cells resuspension. In that case, add 204 µL of the staining mix to each tube of cells.

11.2.4 Incubate at room temperature for 30 to 60 minutes, protected from light.

11.3 Analyze cells by fluorescence microscope:

11.3.1 If cells are on cover slips, invert cover slip on a glass slide.

Δ Note: you can add a drop of anti-fading solution and before inverting cover slip onto glass slide. Edges can be sealed with rubber cement or clear nail polish to ensure cells do not dry out.

11.3.2 Analyze cells by fluorescence microscopy using the appropriate filters as soon as possible (annexin V-iFluor 488: green channel; propidium iodide: TRITC channel).

12. Data Analysis

FOR FLOW CYTOMETRY

The following are general guidelines. Specific methods of analysis will vary with different flow cytometer analysis programs.

- Establish appropriate FSC vs SSC gates to exclude debris and cell aggregates.
- Collect Annexin V conjugate and PI (or viability dye used) fluorescence in the appropriate channels.
- Using fluorescence intensity, determine fold change between control and treated cells.

FOR FLUORESCENCE MICROSCOPY

- We recommend acquiring several images per well.
- We recommend data analysis after coding and mixing images to ensure unbiased results.
- For manual analysis, if you do not have a specific software installed in your microscope, you can download ImageJ, an open source image processing designed for scientific multidimensional images by the National Institute of Health (NIH).

The table below can be used as guidance for interpretation of results:

	↓ Annexin V	↑ Annexin V
↓ Propidium iodide	No apoptosis / viable cells	Early apoptosis
↑ Propidium iodide	Dead cells	Late apoptosis / necrotic cells

Δ Note: elevated Annexin V signal and/or PI staining: apoptosis is an ongoing process so that cells stained with Annexin V should not be kept for long before measurement. Viable cells which still maintain membrane integrity may become positive for PI since the dye can enter intact cells through slow diffusion. Analyze cells as soon as you finish staining.

13. Typical Data

Data provided for **demonstration purposes** only.

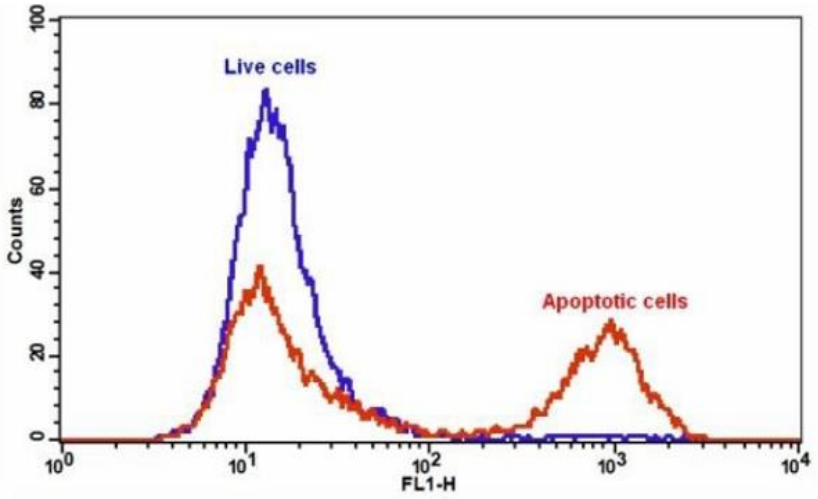


Figure 1. Detection of phosphatidylserine (PS) exposure in Jurkat cells using Annexin V-iFluor 488 Apoptosis Detection Kit (ab219916). Jurkat cells were left untreated (blue) or treated with 20 µM camptothecin (red) in a 37°C, 5% CO_2 incubator for 4-5 hours. Cells were then incubated with Annexin V-iFluor reagent and PI for 30 minutes. The fluorescence intensitiy of Annexin V-iFluor 488 was measured with a FACSCalibur (BD Systems) flow cytometer using the FL1channel.

In live non-apoptotic cells, Annexin V-iFluor 488 conjugate detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells Annexin V-iFluor 488 conjugate binds to phosphatidylserine, which is located on the outer leaflet of the cell membrane, resulted in increased staining intensity.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)