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ab214484
Annexin V – DY-634/ PI Apoptosis Detection Kit

For the quantitative detection of phosphatidylserine (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 – DY-634/ PI Apoptosis Detection Kit (ab214484) contains Annexin V labeled with DY-634 (red fluorescence), which allows the identification and quantitation of apoptotic cells on a single-cell basis by flow cytometry. Simultaneous staining of cells with Annexin V – DY634 (red fluorescence) and the non-vital dye propidium iodide (PI) (orange fluorescence) allows the discrimination of intact cells (AnnexinV-DY-634 negative, PI Staining Solution negative), early apoptotic (Annexin V-DY-634 positive, PI Staining Solution negative) and late apoptotic or necrotic cells (Annexin V-DY-634 positive, PI Staining Solution positive).

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.

2. Protocol Summary

Induce apoptosis in cells using the desired method

Resuspend in 1 X Annexin-binding buffer

Add 5 µL of the Annexin V-DY634 and 5 µL of PI
to each 100 µL of cell suspension

Incubate cells at RT for 15 min in the dark

After incubation, add 400 µL of 1X Annexin-binding buffer

Analyze cells by flow cytometry

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 in the dark at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt.

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

- Apoptosis detection 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 Condition (Before use)	Storage Condition (After use)
Annexin V DY-634 Conjugate (in PBS, pH 7.4)	500 µL	+4°C	+4°C
Propidium Iodide Staining Solution (in PBS, pH 7.4)	500 µL	+4°C	+4°C
10X Binding Buffer Concentrate	50 mL	+4°C	+4°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.
- MilliQ water or other type of double distilled water (ddH_2O)
- Phosphate-buffered saline (PBS).
- Pipettes and pipette tips, including multi-channel pipette.
- Tubes for the preparation of reagents and buffer solutions

8. Technical Hints

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay. 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 components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Annexin V-DY-634 Conjugate:

Ready to use as supplied. Mix conjugate thoroughly by pipetting up and down prior to use to ensure a homogenous solution. Keep on ice while in use. Store at 4°C in the dark.

9.2 Propidium Iodide Staining Solution:

Ready to use as supplied. Mix solution thoroughly by pipetting up and down prior to use to ensure a homogenous solution. Keep on ice while in use. Store at 4°C in the dark.

9.3 10X Binding Buffer Concentrate:

Dilute 10X Binding Buffer Concentrate in ddH_2O to create 1X Binding Buffer Solution. Prepare only as much solution as is needed on the day of the experiment. Equilibrate 1X Binding Buffer Solution at room temperature. Store remaining 10X Binding Buffer Concentrate at 4°C.

Δ Note: Diluted 1X Binding Buffer Solution can be stored at 4°C for up to 1 month.

10. Assay Procedure

- Prepare all reagents as directed in the previous sections.
- We recommend that you assay all controls and samples in duplicate.
- The reagents (Annexin V DY-634 conjugate and Propidium Iodide solution) 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 controls:

- Jurkat cells treated with 6 µM camptothecin for 4 hours.
- MN-cells treated with 200 µM H_2O_2 for 6 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.

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

- Untreated unstained cells.
- Untreated cells stained with Annexin V DY-634 alone (no PI).
- Untreated cells stained with PI alone (no Annexin V – DY634)

10.3 Harvest untreated and treated cells (1 x 10^6 cells/mL).

10.3.1 Suspension cells: transfer cells to collection tube directly.

10.3.2 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.

10.4 Wash cells twice at room temperature in PBS.

10.5 Resuspend cells in 1X Binding Buffer Solution at a final concentration 1 x 10^6 cells/mL.

10.6 To each 100 µL of cell suspension, add 5 µL of Annexin V-DY-634 Conjugate and 5 µL of Propidium Iodide Staining Solution.

10.7 Incubate cells at room temperature for 15 minutes in the dark.

10.8 Add 400 µL of 1X Binding Buffer Solution.

10.9 Analyze cells by flow cytometry within one hour.

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

10.9.2 Use set up controls (step 10.2) to set up necessary laser compensations.

10.9.3 Collect DY-634 fluorescence in FL3 (Ex/Em max = 635/658 nm) and PI fluorescence in FL2 (Ex/Em max = 493/636 nm).

11. Data Analysis

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

11.1 Establish appropriate FSC vs SSC gates to exclude debris and cell aggregates.

11.2 Collect Annexin V-DY-643 and Propidium Iodide fluorescence in the appropriate channels (typically, FL3 and FL2 respectively).

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

	↓ Annexin V-DY-634	↑ Annexin V-DY-634
↓ Propidium Iodide	No apoptosis / viable cells	Early apoptosis
↑ Propidium Iodide	Dead cells	Late apoptosis / necrotic cells

Δ Note: Elevated Annexin V – DY-634 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.

12. Typical Data

Data provided **for demonstration purposes only.**

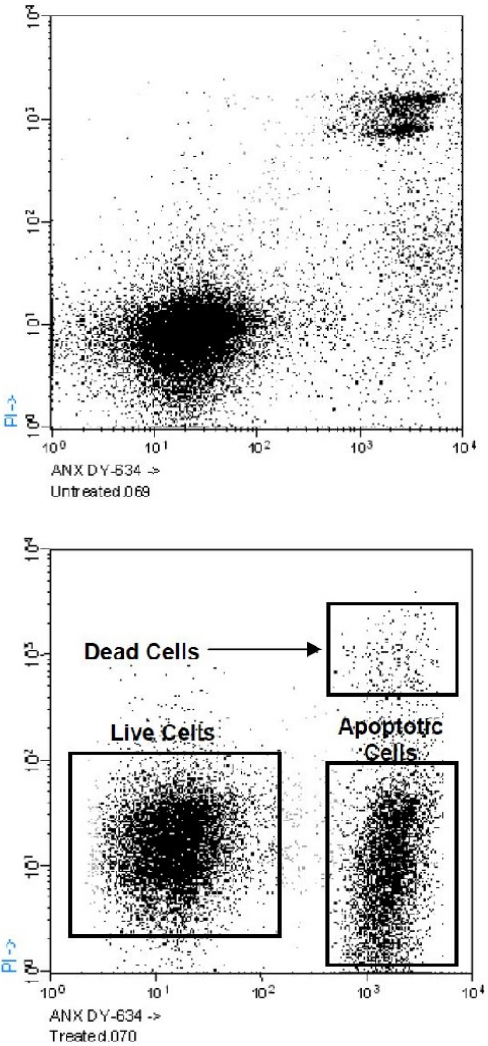

Figure 1. Jurkat cells (human T-cell leukemia cell line) were analyzed with Annexin V – DY-634/ PI Apoptosis Detection Kit (ab214484). Top panel shows untreated cells; cells treated with 6 µM camptothecin for four hours are shown in the bottom panel. Gating showing the different populations of live, dead and apoptotic cells are shown in the figure.

13. FAQs

Q: As part of my protocol, I have to fix my cells with 1% methanol. I can't see much signal. What is the problem?

A: For annexin V staining, it is not necessary to fix cells at all. If you are performing other staining with the same subset of cells and you have fixed your cells with 1% PFA or methanol, there can be some signal quenching. This is likely due to excessive amount of $CaCl_2$ present in the binding buffer (calcium ions are needed for the binding of Annexin V to membrane). To avoid this issue, use the provided 10X Binding Buffer (diluted to 1X) for fixation.

Q: I have run out of 10X Binding Buffer and I would like to make more. Could you tell me the composition?

A: Yes, the 10X Binding Buffer is made of 0.1 M Hepes/NaOH (pH 7.4), 1.4 M NaCl and 25 mM $CaCl_2$.

14. Notes

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