ELISA PRODUCT INFORMATION & MANUAL

Annexin V Apoptosis Detection Kit

NBP2-54875

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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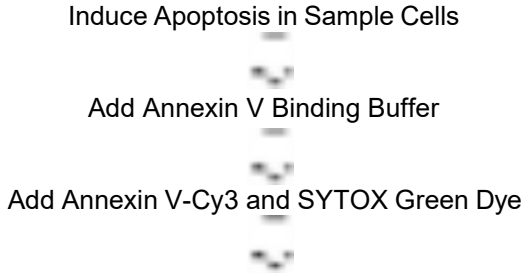
1. Overview

The assay is based on the observation that soon after initiating apoptosis, cells translocate the membrane phospholipid phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface. Once on the cell surface, PS can easily be detected by staining with a fluorescent conjugate of Annexin V, a protein that has a strong natural affinity for PS. The one-step staining procedure takes only 10 minutes. In addition, the assay can be directly performed on live cells, without the need of fixation.

Novus Biologicals's Annexin Annexin V Apoptosis Detection Kit with SYTOX includes Annexin V-Cy3, SYTOX green dye, and binding buffer. The SYTOX green dye is impermeant to live cells and apoptotic cells, but stains necrotic cells with intense green fluorescence by binding to cellular nucleic acids.

After staining a cell population with Annexin V-Cy3 and SYTOX Green dye in the provided binding buffer, apoptotic cells show red fluorescence, dead cells show green fluorescence and live cells show little or no fluorescence. These populations can easily be distinguished by Fluorescence microscopy using FITC and rhodamine filters or by flow cytometry using the FL1 channel (Ex. 488 nm/Em. 530 nm) for SYTOX Green dye and FL2 channel for Annexin V-Cy3 (Ex. 543 nm/Em. 570 nm).

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Annexin V-Cy3	500 µL
SYTOX Green Dye	100 µL
1X Binding Buffer	50 mL

* Store at +4°C. Do not freeze.

- Thaw the SYTOX Green dye in room temperature before use.
- Stable for one year under proper storage conditions.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Flow Cytometer

4. Assay Protocol

1. Incubation of cells with Annexin V-Cy3:

a) Induce apoptosis by desired methods. Concurrently incubate a control culture *without* induction.

b) Collect 1-5 x 10^5 cells by centrifugation.

c) Re-suspend cells in 500 µl of 1X Binding Buffer

d) Add 5 µl of Annexin V-Cy3 and 1 µl of SYTOX Green dye.

For ***adherent cells***, gently trypsinize and wash cells once with serum-containing media *before* incubation with Annexin V-Cy3 and SYTOX dye.

e) Incubate at room temperature for 5 min in the dark.

2. Quantification by Flow Cytometry:

Analyze the stained cells by flow cytometry using FL1 channel for SYTOX Green dye (Ex = 488 nm; Em = 530 nm) and FL2 channel for Annexin V-Cy3 (Ex = 543 nm; Em = 570 nm).

The cell population should separate into three groups: live cells with only a low level of fluorescence, apoptotic cells with red fluorescence and necrotic cells with green fluorescence.

Note:

The flow cytometric results can also be confirmed by viewing the cells under a fluorescence microscope using FITC filter for SYTOX and rhodamine filter for Annexin V-Cy3.

5. Troubleshooting

Problem	Reason	Solution
High Background	Celldensity is higher than recommended	Refer to datasheet and use the suggested cell number
	Increased volumes of components added	Use calibrated pipettes accurately
	Incubation of cell samples for extended periods	Refer to datasheets and incubate for exact times
	Use of extremely confluent cells	Perform assay when cells are at 80-95% confluency
	Contaminated cells	Check for bacteria/ yeast/ mycoplasma contamination

Problem	Reason	[redacted]	Solu
Lower signal levels	Washing cells with	[redacted]	Alwa bindir for
	PBS		wash
	fixation cells)		
	Cells did not initiate apoptosis		Deter time- initiat apopt induc (time- exper
	Very few cells used for analysis		Refer sheet appro cell
	Incorrect setting of the equipment		Refer datas

	used to read samples	[redacted]	use t recom filter
	Use of expired kit or improperly stored reagents		Alwa the e and s comp appro

Problem	Reason	Solution
Erratic results	Uneven number of cells seeded in the wells	Seed only healthy cells (correct passage number)
	Adherent cells dislodged at the time of experiment	Perform experiment gently and in duplicates or triplicates for each treatment
	Incorrect incubation times or temperatures	Refer to datasheet & verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
	Increased or random staining observed in adherent cells	Always stain cells with Annexin before fixation (makes cell membrane leaky)