



Annexin V-Cy3 Apoptosis Detection Kit Plus

Manufactured by BioVision.

ALX-850-257-KI01: ~25 tests
ALX-850-257-KI02: ~100 tests

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For laboratory use only. Not for human or diagnostic use.

I. Introduction:

The **Annexin V-Cy3 Apoptosis Detection Kit Plus** is based on the observation that soon after initiating apoptosis, most cell types 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. The Annexin V-Cy3 Apoptosis Detection Kit Plus 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).

II. Kit Contains:

Components	850-257-KI01	850-257-KI02
	25 tests	100 tests
Annexin V-Cy3	125 µl	500 µl
SYTOX Green Dye	25 µl	100 µl
Binding Buffer	2.5 ml	50 ml

III. Annexin V-Cy3 Assay Protocol:

1. Induce apoptosis by desired method. Concurrently incubate a control culture *without* induction.
2. Collect $1-5 \times 10^5$ cells by centrifugation.
3. Resuspend cells in 500 µl of 1x Binding Buffer.
4. Add 5 µl of Annexin V-Cy3 and 1 µl of SYTOX Green dye
Note: Thaw the SYTOX Green dye in room temperature before use.
5. Incubate at room temperature for 5-10 minutes in the dark.
6. 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.

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.

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

IV. Storage:

- Store kit at +4°C.