



## **Annexin V-FITC Apoptosis Detection Kit Plus**

*Manufactured by BioVision.*

**ALX-850-255-KI01: ~25 tests**  
**ALX-850-255-KI02: ~100 tests**

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

## I. Introduction:

The **Annexin V-FITC 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-FITC Apoptosis Detection Kit Plus includes V-FITC, 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-FITC and SYTOX green dye in the provided binding buffer, apoptotic cells show green fluorescence, dead cells show a higher level of green fluorescence and live cells show little or no fluorescence. These populations can easily be distinguished using a flow cytometry with the 488 nm line of an argon-ion laser for excitation. Both annexin V-FITC and SYTOX green dye emit green fluorescence that can be detected in the FL1 channel, freeing the other channels for the addition of other probes in multi-color labelling experiments.

## II. Kit Contents:

Components	850-255-KI01	850-255-KI02
	25 tests	100 tests
Annexin V-FITC	125 µl	500 µl
SYTOX Green Dye	25 µl	100 µl
Binding Buffer	12.5 ml	50 ml

## III. Annexin V-FITC Plus 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 500µl of 1x Binding Buffer.
4. Add 5µl of Annexin V-FITC and 1µl of SYTOX Green Dye  
Note: Thaw the SYTOX Green Dye at room temperature before use.
5. Incubate at room temperature for 5-10 minutes in the dark.
6. Analyze the stained cells by flow cytometry (Ex = 488 nm; Em = 530 nm).

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

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

## IV. Storage and stability:

- Store kit at +4°C.