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ANNEXIN V-EGFP
APOPTOSIS DETECTION KIT

Code: 310-BV-9A5A,B,C ***Lot No***

Exp.: 1 year from date of dispatch

Introduction: The Annexin V-EGFP Apoptosis Detection Kit 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 be easily detected by staining with a fluorescent conjugate of Annexin V, a protein that has a strong natural affinity for PS (1,2). The one-step staining procedure takes only 10 minutes. In addition, the assay can be directly performed on live cells, without the need for fixation. Detection can be analyzed by flow cytometry or by fluorescence microscopy with a FITC filter. EGFP is brighter and much more photo-stable than other fluorescent reagents.

Kit Contents:

Components	310-BV-9A5A	310-BV-9A5B	**310-BV-9A5C**
	25 assays	100 assays	**400 assays**
Annexin V-EGFP	125µl	500µl	**2 ml**
1X Binding Buffer	12.5 ml	50ml	**2x100 ml**
Propidium Iodide (PI)	125µl	500µl	**2 ml**

Annexin V-EGFP Assay Protocol:

A. Incubation of cells with Annexin V-EGFP

1. Induce apoptosis by desired method.
2. Collect 1-5 x 10^5 cells by centrifugation.
3. Resuspend cells in 200 µl of 1X Binding Buffer.
4. Add 5 µl of Annexin V-EGFP and 5 µl of propidium iodide (PI, optional.)
5. Incubate at room temperature for 5 min in the dark.

Proceed to B or C below depending on method of analysis.

B. Quantification by Flow Cytometry

Analyze Annexin V-EGFP binding by flow cytometry (Ex = 488 nm; Em = 530 nm) using FITC signal detector (usually FL1) and PI staining by the phycoerythrin emission signal detector (usually FL2).

For adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-EGFP (A.3-5).

C. Detection by Fluorescence Microscopy

Place the cell suspension from Step A.5 on a glass slide. Cover the cells with a glass coverslip.

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For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (A.5), invert coverslip on a glass slide and visualize cells. The cells can also be washed and fixed in 2% formaldehyde before visualization. (Cells must be incubated with Annexin V-EGFP before fixation since any cell membrane disruption can cause nonspecific binding of Annexin V to PS on the inner surface of the cell membrane.) Observe the cells under a fluorescence microscope using a dual filter set for FITC & rhodamine.

Cells which have bound Annexin V-FITC will show green staining in the plasma membrane. Cells which have lost membrane integrity will show red staining (PI) throughout the nucleus and a halo of green staining (EGFP) on the cell surface (plasma membrane).

References:

*1.Koopman, G., et al. (1994) Blood **84**:1415-1420.*
***2.** Martin, S. J., et al. (1995) J. Exp. Med. **182**:1545-1556*

Storage: Store kit at +4°C. All reagents are stable for one year under proper storage conditions.

For Research Use Only. Not For Diagnostic or Therapeutic Use.

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