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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	125μΙ	500μΙ	2 ml
1X Binding Buffer	12.5 ml	50ml	2x100 ml
Propidium Iodide (PI)	125μΙ	500μΙ	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<sup>5</sup> 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 (Pl, optional.)
- 5. Incubate at room temperature for 5 min in the dark.

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

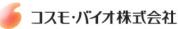
## B. Quantification by Flow Cytometry

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.

<u>Conditions:</u> The information disclosed herein is not be construed as a recommendation to use the above product in violation of any patents. ImmunoKontact will not be held responsible for patent infringement or other violations that may occur with the use of our products.

<u>Caution:</u> Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive compounds in plumbing.

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