For research use only

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MBL™ International Corporation

Annexin V-EGFP Apoptosis Detection Kit

This kit is intended for research use only. Not for use in diagnostic procedure.

1. 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 an enhanced green fluorescent protein (EGFP) fusion 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.

2. KIT CONTENTS:

Code No. BV-K104-2 : 25 assays BV-K104-3 : 100 assays BV-K104-4 : 400 assays

Materials	Quantity					
	25 assays		100 assays		400 assays	
Annexin V-EGFP	125µL	1 vial	500µL	1 vial	2mL	1 vial
1x Binding Buffer	12.5mL	1 vial	50mL	1 vial	100mL	2 vials
Propidium Iodide (PI)	125µL	1 vial	500µL	1 vial	2mL	1 vial

3. Annexin V-FITC 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 500 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

1. Place the cell suspension from Step A.5 on a glass slide. Cover the cells with a glass coverslip.
   For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (A.5), invert coverslip on 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.)
2. Observe the cells under a fluorescence microscope using a dual filter set for FITC & rhodamine.
   Cells which have bound Annexin V-EGFP 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).

4. STORAGE CONDITIONS:

All kit components must be stored at 2~8°C.
All reagents are stable for one year after manufacturing when stored at 2~8°C.

5. RELATED PRODUCTS:

1. MitoCapture™ Mitochondrial Apoptosis Detection Kit
2. Annexin V-FITC, -Cy3, -Cy5, -EGFP, -Biotin, -PE Kits & Bulk Reagents
3. CaspSCREEN™ Flow Cytometric Apoptosis Detection Kit
4. Caspase-1,-2,-3,-4,-5,-6,-8,-9,-10 Activity Assay Kits
5. Active Recombinant Caspase-1,-2,-3,-4,-5,-6,-7,-8,-9,-10 & Procaspase-3
6. Ready-to-use Caspase Substrates and Inhibitors and Sets
7. THP-1 and Jurkat Cell Lysates, Induced and Uninduced
8. Anti-Caspase-1,-2,-3,-4,-5,-6,-7,-8,-9,-10,-11,-12,-13,-14 & Active Casp-3,-9
9. Quick Apoptotic DNA Ladder Detection Kit
10. TUNEL-based kits for *In Situ* Detection of Apoptosis
11. Ready-to-use Apoptosis Inducers and Set
12. Quick Cell Proliferation Assay Kits