Web: www.ImmunoK.com
Email: IKinfo@amsbio.com

ANNEXIN V-EGFP REAGENT

Code: 210-BV-3A5C ***Lot no.:***

Quantity: 1000 assay (1.0 ml) ***Exp:*** 1 year from date of dispatch

Description: Annexin V is a reagent for detecting the early stages of appoptosis. During apoptosis, phosphatidylserine (PS) is translocated from the cytoplasmic face of the plasma membrane to the cell surface. Annexin V has a stong,Ca^{2+} - dependent affinity for PS and therefore is used as a probe for detecting apoptosis. . The annexin V-EGFP fusion is brighter and photo-stable, ideal for detecting apoptosis by fluorescence microscopy with a FITC filter or by flow cytometry.

Assay Protocol:

A. Incubation of cells with Annexin V- EGFP:

1. Induce apoptosis by desired methods.
2. Collect 1 x 10^5 cells by centrifugation.
3. Resuspend cells in 500 µl of 1X Annexin V Binding Buffer (Product Code 200-BV-0A5A, 100 ml).
4. Add 1 ul of Annexin V-EGFP and 1 µl of propidium iodide.
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, trypsinize and gently wash cells with serum-containing medium before incubation with Annexin V-EGFP (step A.3-5).

C. Detection by Fluorescence Microscopy:

1. Place the cell suspension from Step A.5 on a glass slide, and cover with a glass coverslip. For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (step A.5) invert coverslip on a glass slide and visualize cells. The cells can also be washed with 1X Annexin V Binding Buffer and fixed in 2% formaldehyde before visualization. (Cells must be incubated with Annexin V-EGFP before fixation because 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 and 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).

Storage: Annexin V reagent should be stored at +4 °C.

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

Conditions: 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.

Rev. 09/04

UNITED KINGDOM, INTERNATIONAL:
AMS BIOTECHNOLOGY (EUROPE) LTD.
63B MILTON PARK, ABINGDON OXON OX14 4RX
TEL: +44 (0) 1235 828200
FAX: +44 (0) 1235 820482

SWITZERLAND:
AMS BIOTECHNOLOGY (EUROPE) LTD.
CENTRO NORD-SUD 2E, CH-6934 BIOGGIO (LUGANO)
TEL: +41 (0) 91 6045522
FAX: +41 (0) 91 6051785

DEUTSCHLAND:
AMS BIOTECHNOLOGY GMBH
HENKELLSTRASSE 15, D-65187 WIESBADEN
TEL: +49 (0) 69 779099
FAX: +49 (0) 69 13376880