APOPTEST™-BIOTIN (cat.no.: B700)

Annexin V-Biotin Kit: *500 µl Annexin V-Biotin solution*
10 ml 10 x concentrated buffer
1 ml with 250 µg/ml Propidium Iodide

Contents
1 vial containing 500 µl Annexin V-Biotin solution.
6 vials containing 1.7 ml 10x concentrated binding buffer.
1 vial containing 1 ml with 250 µg/ml propidium iodide

Features of Annexin V-Biotin

Annexin V-Biotin	:	1:8 stoichiometric complex.
Purity	:	> 99% pure according to Fast Protein Liquid Chromatography.
Quality	:	> 99% of the protein has full phospholipid binding properties according to ellipsometry.
Quantity	:	500 µl Annexin V-Biotin solution.
Concentration	:	100 µg/ml
Additives	:	None.

Biological properties
Annexin V-Biotin binds to phosphatidylserine.

Storage of Annexin V-Biotin solution and the 10 fold concentrated buffer
Store in the dark and refrigerated at 2-8^{o}C.

Storage of the propidium iodide.
Store in the dark and refrigerated at 2-8^{o}C.

Application
The APOPTEST™-BIOTIN is designed to measure quickly Apoptosis in a variety of adherent cell types in culture and in tissues *in vitro* and *in vivo*.
APOPTEST™-BIOTIN can be applied to samples derived from mammals, avian and insects.

APOPTEST™-BIOTIN is to be used *in vitro* for research purposes only, not for diagnostic or therapeutic procedures!

Examples of APOPTEST™-BIOTIN Protocols

1 Detection of apoptosis of adherent cell types in culture.

1.1 *Bicolour analysis using Annexin V-Biotin, streptavidin-FITC and propidium iodide.*

- Culture the cells of interest in the wells of a 24 wells plate.
- Induce apoptosis according to your specific protocols.
- Add 5 µl of Annexin V-Biotin stock solution to 500 µl culture medium at the time point of analysis. (*Annexin V-Biotin binds optimally to apoptotic cells at free Ca^{2+} levels of more than 1.5 mM. Should the medium contain less, adjust the level by adding $CaCl_2$ or replace the medium by 1x binding buffer. Our experience is that RPMI1640 is less suitable for the assay. Other media like DMEM are recommended.*).
- Incubate the cells for at least 5 minutes with Annexin V-Biotin. (*The incubation temperature may vary from 0° C to 37° C. Please be aware that Annexin V-Biotin is unstable at temperatures above 42° C.*).
- Remove the supernatant containing the detached cells from the well.
- Wash the detached cells twice with culture medium or 1x binding buffer to remove unbound Annexin V-Biotin. (*At Ca^{2+} levels of more than 1.5 mM bound Annexin V-Biotin hardly desorbes from the surface.*).
- Rinse the adherent cells in the wells twice with culture medium or 1x binding buffer before harvesting.
- Harvest by scraping with a rubber policeman. (*Methods of harvesting using trypsin and EDTA reduces the amount of cellular bound Annexin V-Biotin and may induce viable cells to expose PS. The extent to which this occurs depends on the cell type.*).
- Dilute the cells with culture medium or 1x binding buffer to 10^5-10^6 cells/ml.
- Add streptavidin-FITC and propidium iodide (2.5 µg/ml final concentration) to the cell sample
- Incubate for 15 minutes on ice before flow cytometric analysis.
- The two colour dot plot will show three distinct populations. i) the viable cells which were not damaged during collection by scraping. These cells have low FITC and low PI signal, ii) the damaged viable cells, which have low FITC and high PI signal and iii) the apoptotic cells, which have high FITC and low PI signal. Depending on the cell type and the fraction (adherent or detached) a fourth population may be present. iv) the secondary necrotic cells with high FITC and high PI signal.

1.2 *Tricolour analysis using Annexin V-Biotin, streptavidin-FITC, a PE-labelled antibody against an intracellular antigen of choice and propidium iodide.*

- The protocol of this analysis is identical to 1.1. up to resuspension of the Annexin V-Biotin labelled cells at a density of 10^5-10^6 cells/ml.
- Instead, fix the cells by resuspending the cell pellet by incubating for 5 minutes in methanol of -20°C.
- Wash the cells once with PBS and once with PBS, containing 1 mg/ml BSA.
- Add to 100 µl resuspended cell sample FITC-labelled streptavidin and a PE-labelled antibody against an antigen of choice.
- Incubate for 1 hour at room temperature.
- Wash twice with PBS/BSA and finally resuspend the cells in PBS,

supplemented with 2.5 µg/ml propidium iodide and 100 µg/ml RNAse.
- Incubate for 15 minutes on ice before flow cytometric analysis.
- This protocol enables you to study the expression of an intracellular antigen of choice during the process of apoptosis.
- If you have FITC-labelled antibodies you can make use of streptavidin-PE for the tricolour analysis.

2 Detection of apoptosis of suspended cell types in culture.

2.1 *Tricolour analysis using Annexin V-Biotin, streptavidin-FITC, a PE-labelled antibody against an intracellular antigen of choice and propidium iodide.*

- Apoptosis of suspended cells can be easily measured using APOPTEST™-FITC (Annexin V-FITC). However, if you wish to do tricolour analysis while using methanol for fixation, Annexin V-FITC is not recommended because of the loss of FITC signal during fixation.
- Resuspend the cells in 10x diluted binding buffer to 10^5-10^6 cells/ml.
- Add 5 µl Annexin V-Biotin to 500 µl cell sample and incubate for 5 min on ice.
- Wash twice with diluted binding buffer to remove unbound Annexin V-Biotin.
- Fix the cells by resuspending the cell pellet in methanol of -20^{o}C and incubating for 5 minutes.
- Wash the cells once with PBS and once with PBS, containing 1 mg/ml BSA.
- Add to 100 µl resuspended cell sample FITC-labelled streptavidin and a PE-labelled antibody against an antigen of choice.
- Incubate for 1 hour at room temperature.
- Wash twice with PBS/BSA and finally resuspend the cells in PBS, supplemented with 2.55 µg/ml propidium iodide and 100 µg/ml RNAse.
- Incubate the sample 15 minutes on ice before flow cytometric analysis.
- This protocol enables you to study the expression of an intracellular antigen of choice during the process of apoptosis.
- If you have FITC-labelled antibodies you can make use of streptavidin-PE for the tricolour analysis.

3 Detection of Apoptosis in tissues.

- Apoptosis of cells in tissues, which are either cultured or freshly isolated from human or animal, can be detected using Annexin V-Biotin.
- Emerge the tissue in a small volume of culture medium or 1x binding buffer. If you are using culture medium, the Ca^{2+} level should be 1.5 mM or more.
- Add 10 µl Annexin V-Biotin stock solution to 100 µl medium.
- Incubate for 30 minutes at room temperature or 37^{o}C.
- Wash the tissue twice with culture medium or 1x binding buffer for 5 minutes each time.
- Fix the tissue in formaldehyde and embed in paraffin.
- Cut thin sections and visualise cell bound Annexin V-Biotin in the section by performing standard procedures to stain for biotin-labelled compounds in tissue sections.
- This protocol enables you to visualise early and late apoptotic cells in tissues.
- If you wish to quantitate, incubate the tissue section with radiolabelled streptavidin. This will provide semi-quantitative information as to the extent of

apoptosis in a certain section and not the number of apoptotic cells.

- Please note that the boundaries of the tissue may bind Annexin V-Biotin because of damage to the viable cells at these edges during tissue isolation. It is recommended to analyse sections, which do not contain these boundaries.