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Product Information

Apoptosis and Necrosis Quantitation Kit

Catalog Number: 30017

Unit Size: 50 assays

Kit Contents
- FITC-Annexin V in TE, pH 7.5/0.1% BSA/0.1% NaN_3, 250 uL (component 99903)
- Ethidium Homodimer III (EthD-III) in PBS, 250 uL (component 99904)
- 5X Annexin V Binding Buffer, 15 mL (component 99902)

Storage and Handling
Store the kit at 4ºC. Do not freeze. Protect FITC-Annexin V and Ethidium Homodimer III from light. When stored as directed, the kit is stable for at least 6 months from the date it is received.

Product Description
Apoptosis and necrosis are two processes by which cells die. Apoptosis is an active, regulated disassembly of the cell from within. During apoptosis, phosphatidylserine (PS) is translocated from the inner to the outer surface of the cell, allowing the dying cell to be engulfed by phagocytic cells. Annexin V is a 35 kD Ca^{2+}-dependent phospholipid binding protein with a high affinity for PS. The Apoptosis, Necrosis and Healthy Cells Quantitation Kit features Annexin V labeled with fluorescein (FITC) (excitation/emission: 492/514 nm) for staining PS on the surface of apoptotic cells with green fluorescence.

Necrosis normally results from a severe cellular insult. Both internal organelle and plasma membrane integrity are lost, resulting in spilling of cell contents into the surrounding environment. Ethidium Homodimer III (EthD-III) is a highly positively charged nucleic acid probe, which is impermeant to live cells and early apoptotic cells, but stains necrotic cells and late apoptotic cells with red fluorescence (excitation/emission bound to DNA: 528/617 nm). EthD-III is a superior alternative to propidium iodide (PI) or Ethidium Homodimer I due to its significantly higher affinity for DNA and higher fluorescence quantum yield.

Apoptosis and Necrosis Quantitation Kit provides a convenient assay for detecting apoptotic (green) and necrotic (red) cells within the same cell population by flow cytometry or fluorescence microscopy. For more information, please see Frequently Asked Questions, next page.

Please also see our Apoptosis, Necrosis & Healthy Cell Quantitation Kit, which includes the membrane permeant nuclear stain Hoechst 33342, which stains the nuclei of all cells with blue fluorescence. Biotium's Apoptosis and Necrosis Quantitation Kit Plus and Apoptosis, Necrosis & Healthy Cell Quantitation Kit Plus feature CF™488A-Annexin V, which is brighter and more photostable than FITC-Annexin V.

Assay Protocols
Note: We recommend that you include two control samples, for staining with each of the probes (FITC-Annexin V and EthD-III) separately.

Suspension cells
1. Prepare 1X Binding Buffer by diluting 5X Annexin V Binding Buffer 1:5 with dH_2O.
2. Wash cells with PBS.
3. Resuspend cells at 2-3x10^6 cells /mL in 1X Binding Buffer.
4. Pipet 100 uL cell suspension into a microcentrifuge tube.
5. Add 5 uL of FITC-Annexin V and 5 uL of EthD-III to each tube.
   Note: reducing the concentration of EthD-III may result in better signal:background ratio for some cell types.
6. Incubate at room temperature for 15 minutes in the dark.
7. For flow cytometry analysis, add 400 uL 1X Binding Buffer to each tube and measure fluorescence in FITC and propidium iodide channels within 1 hour of staining.
8. For fluorescence microscopy analysis, wash cells with 1X Binding Buffer, resuspend cells in 1X Binding Buffer, and observe fluorescence using FITC and Texas Red® filter sets.

Assay protocols, continued

Adherent cells for fluorescence microscopy
1. Prepare 1X Binding Buffer by diluting 5X Annexin V binding buffer 1:5 with dH_2O.
2. Wash cells twice with PBS.
3. Prepare staining solution by adding 5 uL of FITC-Annexin V and 5 uL of EthD-III to 100 uL 1X binding buffer. Prepare enough staining solution to cover cells.
   Note: reducing the concentration of EthD-III may result in better signal:background ratio for some cell types.
4. Incubate samples 15 minutes at room temperature, protected from light
5. Wash cells with 1X Binding Buffer 1-2 times.
6. Cover cells with 1X Binding Buffer and observe fluorescence using FITC and Texas Red® filter sets.

Adherent cells for flow cytometry
1. Detach cells from cell culture plate or well using trypsin or other cell dissociation method.
2. Pellet cells and discard supernatant.
3. Follow staining protocol for suspension cells.

Optional: formaldehyde fixation may be performed for long term preservation of cell staining. Annexin V binding to PS requires calcium, therefore buffers used for washing and fixation should contain 1.25 mM calcium chloride ($CaCl_2$). Fixation may increase background staining by Ethidium Homodimer III. Cells should be washed thoroughly prior to fixation to remove unbound dye.

Expected Results
Green fluorescent plasma membrane staining identifies apoptotic cells, while necrotic cells are identified by red fluorescent nuclear staining. Late apoptotic cells may show both red and green staining.

References
Martin, SJ, et al. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. J Exp Med. 182(5):1545-56 (1995).

Related Products

Catalog number	Product
99902	5X Annexin V Binding Buffer
30018	Apoptosis, Necrosis & Healthy Cell Quantitation Kit
30065	Apoptosis and Necrosis Quantitation Kit Plus
30066	Apoptosis, Necrosis & Healthy Cell Quantitation Kit Plus
30060	CF™488A Annexin V and 7-AAD Apoptosis Kit
30061	CF™488A Annexin V and PI Apoptosis Kit
30029	NucView™488 Caspase-3 Assay Kit for Live Cells
30067	NucView™488 Caspase-3 Substrate and CF™594 Annexin V Dual Apoptosis Assay Kit
30062	NucView™488 and MitoView™633 Apoptosis Kit
30001	JC-1 Mitochondrial Membrane Detection Kit
30063	CF™488A TUNEL Assay Apoptosis Detection Kit
30064	CF™594 TUNEL Assay Apoptosis Detection Kit

Please visit our website at **www.biotium.com** to view our full selection of CF™ dye bioconjugates, including antibodies, antibody labeling kits, phalloidin, annexin V and α-bungarotoxin, as wells as classic fluorescent nucleic acid dyes and hundreds of other products for life science research.

Apoptosis and Necrosis Quantitation Kits
Frequently Asked Questions

Question/Problem	Answer/Suggestion
Can I use the kits for staining fixed cells or tissues?	No. Fixation, freezing, sectioning, or dissociation of tissues can affect the PS on the outer leaflet and compromise membrane integrity. Both Annexin V and Ethidium Homodimer III rely upon the presence of intact membranes in healthy cells to accurately distinguish healthy cells from apoptotic or necrotic cells. To detect apoptosis in fixed cells and tissues we recommend our TUNEL kits. Currently we are not aware of a fluorescent probe that specifically detects necrotic cells in fixed tissues. Necrotic cells in tissue sections are identified based on morphological criteria.
Can I use the kits for staining live embryos, tissue explants or organotypic slice cultures?	The assays are designed to stain dissociated cells in culture and have not been validated for organ culture. Annexin V staining of early chicken and mammalian embryos in culture has been reported in the scientific literature. For staining of living tissues, the specimen would need to be thin enough to allow exposure of the cells to the 36 kDa Annexin V protein. Also, damage to cell membranes from dissection or sectioning of tissues could result in high background staining.
Can I fix cells after staining?	Yes, cells can be fixed with formaldehyde after staining. Because Annexin V staining is dependent on calcium, all buffers used for washing and fixation should contain 1.25 mM $CaCl_2$. Fixation may increase the background signal from Ethidium Homodimer III. Wash the cells several times to remove unbound Ethidium Homodimer III before fixation.
What species does Annexin V cross-react with?	Annexin V is a 36 kDa protein that binds to the phospholipid phosphatidylserine. Therefore Annexin V binding is not species-specific.
What is the source of your Annexin V?	The Annexin V protein that we use is a recombinant protein made in E. coli.
What is the purpose of the binding buffer?	The binding buffer is an isotonic buffer containing calcium, which is essential for the binding of Annexin V to phosphatidylserine.
I am using fewer cells than suggested in the protocol, should I adjust the amount of CF488A- or FITC-Annexin V and Ethidium Homodimer III in the assay?	No, the concentration of Annexin V and Ethidium Homodimer III should be kept constant regardless of cell number. However, the staining concentrations can be increased or decreased if necessary to optimize staining.
I am having trouble with low signal or high background staining.	The concentration of Annexin V and Ethidium Homodimer III can be increased or decreased if necessary to optimize staining for different cell types.
I am afraid that I'm losing some of my apoptotic cells during processing of cells from an adherent culture, how can I make sure that they are accounted for?	You may wish to process floating and detached cells separately – collect the washes and spin them down and then stain those cells using the protocol for suspension cells. Alternatively you may collect floating cells, detach the adherent cells with trypsin (without EDTA), pool the cells together, and use the suspension cell protocol for staining.