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Revised: April 11, 2011

Product Information

Apoptosis and Necrosis Quantitation Kit Plus

Catalog Number: 30065

Unit Size: 50 assays

Kit Contents
- CF™488A-Annexin V in TE buffer, pH 7.5/0.1% BSA/0.1% NaN_3, 250 uL (component 99967)
- Ethidium homodimer III (EthD-III) in PBS, 250 uL (component 99968)
- 5X Annexin V Binding Buffer, 15 mL (component 99902)

Storage and Handling
Store the kit at 4ºC. Do not freeze. Protect CF™488A-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 Plus features Annexin V labeled with CF™488A (excitation/emission: 490/515 nm) for staining PS on the surface of apoptotic cells with green fluorescence. CF™488A is much brighter and more photostable than traditional green fluorescent dyes like fluorescein.

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.

Assay Protocols
Note: We recommend that you include two control samples, for staining with each of the probes (CF™488A-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 CF™488A-Annexin V and 5 uL of EthD-III to each tube.
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 CF™488-Annexin V and 5 uL of EthD-III to 100 uL 1X binding buffer. Prepare enough staining solution to cover cells.
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 fixation should contain 1.25 mM calcium chloride ($CaCl_2$).

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
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

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