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Product Information

Apoptotic, Necrotic and Healthy Cells Quantitation Kit Plus

Catalog Number: 30066

Unit Size: 50 assays

Kit Contents

Component	Cat. no.	Size
CF®488A Annexin V in TE/0.1% BSA/0.1% NaN_3	99967	250 uL
Ethidium Homodimer III (EthD-III) in PBS	99904	250 uL
Hoechst 33342 in dH_2O	30018C	250 uL
5X Annexin V Binding Buffer	99902	15 mL

Storage and Handling

Store the kit at 4ºC. Do not freeze. Protect CF®488A Annexin V, Ethidium Homodimer III and Hoechst 33342 from light. When stored as directed, the kit is stable for at least 6 months from the date it is received.

Spectral Properties

Hoechst 33342 Ex/Em: 352/462 nm (with DNA)
CF®488A Ex/Em: 490/515 nm
Ethidium homodimer III (EthD-III) Ex/Em: 532/625 nm* (with DNA)
*Ethidium Homodimer III also has a strong UV absorbance peak at 279 nm

Product Description

The Apoptosis, Necrosis and Healthy Cell Quantitation Kit Plus provides a convenient assay for detecting apoptotic cells (green)and necrotic cells (red) within the same cell population by flow cytometry or fluorescence microscopy. The nuclear stain Hoechst is included for staining the entire cell population (live and dead) blue.

Apoptosis and necrosis are two major processes by which cells die. Apoptosis is an active, genetically regulated disassembly of the cell from within. During apoptosis, phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane for phagocytic cell recognition. The human anticoagulant Annexin V is a 35 kDa, calcium-dependent phospholipid-binding protein with high affinity for PS. Annexin V labeled with CF®488A stains apoptotic cells green by binding to PS exposed on the cell surface. CF®488A is spectrally similar to fluorescein (FITC), with much brighter and more photostable 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.

EthD-III is a superior alternative to other red dead cell stains such as propidium iodide (PI) or Ethidium Homodimer I (EthD-I) due to its significantly higher affinity for DNA and higher fluorescence quantum yield, resulting in a brighter signal.

Membrane permeant blue fluorescent nuclear dye Hoechst 33342 is included in the kit for staining the entire cell population.

Assay Protocols

Note: This assay must be used on unfixed cells. Both Annexin V and EthD-III rely upon the presence of intact membranes in healthy cells to accurately distinguish healthy cells from apoptotic or necrotic cells. The dyes cannot be used for live/dead discrimination in fixed cells or tissues, and cannot withstand fixation after staining.

Note: We recommend including single stain controls.

Suspension cell staining

1. Prepare 1X Binding Buffer by diluting 5X Annexin V Binding Buffer with dH_2O (mix 1 volume of 5X buffer with 4 volumes of dH_2O).
2. Wash cells with PBS and resuspend cells at 2-3x10^6 cells/mL in 1X Binding Buffer.
3. Pipet 100 uL cell suspension into a microcentrifuge tube.
4. Add 5 uL of CF®488A Annexin V, 5 uL of EthD-III and 5 uL Hoechst 33342 to each tube.

   Note: Reducing the concentration of EthD-III may result in better signal:background ratio for some cell types.
5. Incubate at room temperature for 15 minutes in the dark.
6. For flow cytometry analysis, add 400 uL 1X Binding Buffer to each tube and measure fluorescence in Pacific Blue™, FITC and PE channels within 1 hour of staining.
7. For fluorescence microscopy analysis, wash cells with 1X Binding Buffer, resuspend cells in 1X Binding Buffer, and observe fluorescence using DAPI, FITC and Texas Red® or Cy®3 filter sets.

Adherent cell staining for fluorescence microscopy

1. Prepare 1X Binding Buffer by diluting 5X Annexin V Binding Buffer with dH_2O (mix 1 volume of 5X buffer with 4 volumes of dH_2O).
2. Wash cells twice with PBS.
3. Prepare staining solution by adding 5 uL of CF®488 Annexin V, 5 uL of EthD-III and 5 uL Hoechst 33342 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 DAPI, FITC and Texas Red® or Cy®3 filter sets.

Staining adherent cells for flow cytometry

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

Related Products

Catalog number	Product
99902	5X Annexin V Binding Buffer
29002-29083	Annexin V CF® Dye Conjugates
29006-29082	Annexin V Near IR CF® Dye Conjugates
29003R-29069R	Annexin V CF® Dye Conjugates, Azide-Free, Lyophilized
30072	NucView® 488 and RedDot™2 Apoptosis and Necrosis Kit
10403	NucView® 488 Caspase-3 Substrate, 1 mM in PBS
10407	NucView® 405 Caspase-3 Substrate, 1 mM in PBS
10408	NucView® 530 Caspase-3 Substrate, 1 mM in PBS
30062	NucView® 488 and MitoView™ 633 Apoptosis Kit
30063	CF®488A TUNEL Assay Apoptosis Detection Kit
30064	CF®594 TUNEL Assay Apoptosis Detection Kit
70055	MitoView™ 633
32002-32013	Live-or-Dye™ Fixable Viability Staining Kits
32010	Live-or-Dye™ NucFix Red Staining Kit
30020	ATP-Glo™ Bioluminometric Cell Viability Assay
30068	ViaFluor® SE 405 Cell Proliferation Kit
30086	ViaFluor® SE 488 Cell Proliferation Kit
30050	ViaFluor® SE CFSE Cell Proliferation Kit

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