Product Information

Apoptotic, Necrotic and Healthy Cells Quantitation Kit Plus

Catalog Number: 30066

Unit Size: 50 assays

Kit Contents

<table>
<thead>
<tr>
<th>Component Name</th>
<th>Cat. No.</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF®488A Annexin V in TE/0.1% BSA/0.1% NaN₃</td>
<td>99967</td>
<td>250 uL</td>
</tr>
<tr>
<td>Ethidium Homodimer III (EthD-III) in PBS</td>
<td>99968</td>
<td>250 uL</td>
</tr>
<tr>
<td>Hoechst 33342 in dH₂O</td>
<td>30018C</td>
<td>250 uL</td>
</tr>
<tr>
<td>5X Annexin V Binding Buffer</td>
<td>99902</td>
<td>15 mL</td>
</tr>
</tbody>
</table>

Storage and Handling

Store the kit at 4°C. 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: 350/461 nm (with DNA)
CF®488A Ex/Em: 490/515 nm
Ethidium homodimer III (EthD-III) Ex/Em: 522/593 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 (green), necrotic (red) and healthy (blue) cells within the same cell population by flow cytometry or fluorescence microscopy.

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 anti-coagulant Annexin V is a 35 kDa, Ca²⁺-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 propidium iodide (PI) or Ethidium Homodimer I due to its significantly higher affinity for DNA and higher fluorescence quantum yield.

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

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF® dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

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Cy Dye is a registered trademark of GE Healthcare

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 dH₂O.
2. Wash cells with PBS and resuspend cells at 2-3x10⁶ 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®63 filter sets.

Adherent cell staining for fluorescence microscopy
1. Prepare 1X Binding Buffer by diluting 5X Annexin V Binding Buffer with dH₂O.
2. Wash cells twice with PBS.
3. Prepare staining solution by adding 5 uL of CF®488A 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®63 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

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</tr>
</thead>
<tbody>
<tr>
<td>99902</td>
<td>5X Annexin V Binding Buffer</td>
</tr>
<tr>
<td>30072</td>
<td>NucView® 488 and RedDot™2 Apoptosis and Necrosis Kit</td>
</tr>
<tr>
<td>10403</td>
<td>NucView® 488 Caspase-3 Substrate, 1 mM in PBS</td>
</tr>
<tr>
<td>10407</td>
<td>NucView® 405 Caspase-3 Substrate, 1 mM in PBS</td>
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<tr>
<td>10408</td>
<td>NucView® 530 Caspase-3 Substrate, 1 mM in PBS</td>
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<tr>
<td>30062</td>
<td>NucView® 488 and MitoView™ 633 Apoptosis Kit</td>
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<tr>
<td>30063</td>
<td>CF®488A TUNEL Assay Apoptosis Detection Kit</td>
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<tr>
<td>30064</td>
<td>CF®8594 TUNEL Assay Apoptosis Detection Kit</td>
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<tr>
<td>70055</td>
<td>MitoView™ 633</td>
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<tr>
<td>32002-32009</td>
<td>Live-or-Dye™ Fixable Viability Staining Kits (8 color options)</td>
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