



Apoptotic & Necrotic & Healthy Cells Quantification Kit

Catalog Number: 30018 (50 assays)

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Description

Apoptosis and necrosis are two major processes by which cells die. Apoptosis is an active, genetically regulated disassembly of the cell from within. Disassembly creates changes in the phospholipid content of the cytoplasmic membrane outer leaflet. Phosphatidylserine (PS) is translocated from the inner to the outer surface of the cell for phagocytic cell recognition. The human anticoagulant, annexin V, is a 35 kD Ca^{2+} -dependent phospholipid protein with a high affinity for PS. Annexin V labeled with fluorescein (FITC) ($\lambda_{\text{abs}}/\lambda_{\text{em}} = 492/514 \text{ nm}$) can identify apoptotic cells in green by binding to PS exposed on the outer leaflet. Following Annexin V staining, cells may be fixed with 2% formaldehyde for immunohistochemistry if desired.

Necrosis normally results from a severe cellular insult. Both internal organelle and plasma membrane integrity are lost, resulting in spilling of cytosolic and organellar contents into the surrounding environment. Ethidium homodimer III (EtD-III) is a highly positively charged nucleic acid probe, which is impermeant to live cells or apoptotic cells, but stains necrotic cells with red fluorescence ($\lambda_{\text{abs}}/\lambda_{\text{em}} = 528/617 \text{ nm}$). EtD-III is a superior alternative to propidium iodide (PI) or ethidium homodimer I used in some of our competitors' kits due to its significantly higher affinity for DNA and higher fluorescence quantum yield.

Hoechst 33342 is a cell membrane-permeant, minor groove-binding DNA stain that emits bright blue fluorescence upon binding to DNA ($\lambda_{\text{abs}}/\lambda_{\text{em}} = 350/461 \text{ nm}$). It has been used for staining the nuclei of cells.

This kit provides a convenient assay for quantifying apoptotic (green), necrotic (red) cells and healthy (blue only) within the same cell population by flow cytometry or fluorescence microscopy. Hoechst 33342 stains the nuclei of both apoptotic and necrotic cells. However, healthy cells are stained by Hoechst only, not by FITC-Annexin V and EtD-III. Apoptotic cells are stained both green and blue. Necrotic cells are stained both red and blue. Cells stained blue, green and red are dead cells progressing from the apoptotic cell population.

Kit Contents

FITC-Annexin V, one vial, 250 μL in TE buffer containing 0.1% BSA and 0.1% NaN_3 , pH 7.5

Ethidium Homodimer III, one vial, 250 μL , 200 μM in PBS

Hoechst 33342, one vial, 250 μL , 500 $\mu\text{g}/\text{mL}$ in PBS

5X Binding Buffer, one bottle, 10 mL

Caution: Sodium azide and ethidium homodimer III are hazardous substances. Handle with care and dispose properly.

Storage Condition

Store the kit at 4°C and protected from light. Do not freeze!

Experimental Protocol

Suspension cells

1. Induce apoptosis in cells by a desired method.
2. Dilute 5X Binding Buffer 1:5 in distilled water.
3. Wash cells with PBS once and resuspend cells at $2\text{-}3 \times 10^6$ cells /mL in 1X Binding Buffer.
4. Aliquot cells at 100 $\mu\text{L}/\text{tube}$.
5. Add 5 μL of FITC-Annexin V, 5 μL of Ethidium Homodimer III and 5 μL of Hoechst 33342 solutions to each tube. **Note:** We recommend you set up three additional tubes, one for each of the staining dyes (FITC-Annexin V, EtD-III or Hoechst 33342) as controls.
6. Incubate at room temperature for 15 minutes in the dark.

7. For flow cytometry analysis, add 400 μ L 1X Binding Buffer to each tube and analyze the cells by flow cytometry within 1 hour of staining. If cells are to be fixed, wash cells 1-2 times with 1X Binding Buffer and fix with 2% paraformaldehyde in 1X Binding Buffer.
8. For fluorescence microscopy analysis, wash cells 1-2 times with 1X Binding Buffer and place cell suspension on a glass slide and cover with a glass coverslip. If desired, following washing, cells may be fixed with 2% formaldehyde in 1X Binding Buffer.

Adherent cells for microscope viewing

1. Grow cells directly on a coverslip.
2. Induce apoptosis in cells by a desired method.
3. Dilute 5X Binding Buffer 1:5 in distilled water.
4. Wash cells twice with 1X Binding Buffer.
5. Add 5 μ L of FITC-Annexin V, 5 μ L of Ethidium Homodimer III and 5 μ L of Hoechst 33342 into 100 μ L 1X Binding Buffer to make the staining solution.
6. Stain each coverslip with the staining solution for 15 min.
7. Wash cells with 1X Binding Buffer 1-2 times.
8. Mount coverslip onto a slide with 1X Binding Buffer, aspirate residual 1X binding Buffer and seal coverslip with nail polish. Or if desired, cells may be fixed with 2% formaldehyde in 1X Binding Buffer following washing.
9. Observe cell staining under a fluorescence microscope using a multiband filter set for FITC, rhodamine and DAPI, or separate filters.

Adherent cells for flow cytometry analysis

1. Induce apoptosis in cells by a desired method.
2. Dilute 5X Binding Buffer 1:5 in distilled water.
3. Wash cells with PBS twice and detach cells from cell culture plate or well by trypsin or cell dissociating buffer.
4. Centrifuge to discard supernatant and resuspend cells at $2-3 \times 10^6$ cells /mL in 1X Binding Buffer.
5. Aliquot cells at 100 μ L/tube.
6. Add 5 μ L of FITC-Annexin V, 5 μ L of Ethidium Homodimer III and 5 μ L of Hoechst 33342 solutions to each tube. **Note:** We recommend you set up three additional tubes, one for each of the staining dyes (FITC-Annexin V, EtD-III or Hoechst 33342) as controls.
7. Incubate at room temperature for 15 minutes in the dark.
8. Add 400 μ L 1X Binding Buffer to each tube and analyze the cells by flow cytometry within 1 hour of staining. If cells are to be fixed, wash cells 1-2 times with 1X Binding Buffer and fix with 2% paraformaldehyde in 1X Binding Buffer.

References

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