Apoptotic Cell Isolation Kit

(Catalog #K258-30; 30 isolations; Store kit at 4 °C)

I. Introduction:

The Apoptotic Cell Isolation Kit provides a simple and efficient means for isolation of apoptotic cells or removal of dead cells from cell culture or tissue preparations using annexin V/magnetic beads (MagBeads). Annexin V is a Ca^{2+}-dependent phospholipid binding protein with high affinity for phosphatidylserine (PS), which is redistributed from the inner to the outer plasma membrane leaflet in apoptotic or dead cells. Once on the cell surface, PS becomes available for binding to annexin V and any of its conjugates. Binding of annexin V-biotin to apoptotic cells followed by binding of the biotin to streptavidin-MagBeads enables separation of apoptotic cells from living cells. The apoptotic cells bound to the MagBeads adhere to the magnet, while non-apoptotic cells stay in suspension. The separated apoptotic and healthy cells can then be used in a variety of assays to study apoptotic mechanisms and pathways. The kit has also been successfully used to remove dead cells from healthy cell population.

II. Kit Contents:

Component	K258-30 30 Isolations	Color Code	Part Number
Annexin V-Biotin 1X Binding Buffer	150 µl 25 ml	Yellow WM	K258-30-1 K258-30-2
Streptavidin MagBeads	1.5 ml	Brown	K258-30-3
Apoptotic Cell Releasing Buffer	10 ml	NM	K258-30-4

III. General Consideration:

- Read the entire protocol before beginning the procedure.
- Store all components at 4 ◦C..

IV. Apoptotic/Dead Cell Isolation Procedure

1. Induce apoptosis by desired method.
2. Collect 5-10 x 10^6 cells by centrifugation.
3. Resuspend cells in 100 µl of 1X Binding Buffer.
4. Add 5 µl of Annexin V-Biotin, mix gently.
5. Incubate at room temperature for 5-10 min.
6. Centrifuge the cells for 2 min at 600 x g, remove the supernatant.
7. Wash the cells with 200 µl 1X Binding Buffer, repeat Step 6.
8. Resuspend the cells in 200 µl 1X Binding Buffer.
9. Wash the Streptavidin MagBeads with 1X Binding Buffer:
   - Transfer 50 µl/assay of the MagBeads suspension to a new tube.
   - Separate the beads by the magnetic separator (BioVision, Cat. 1999-1) and remove the solvent.
   - Add 200 µl 1X Binding Buffer to the Beads.
   - Separate the Beads again and resuspend them in 50 µl/assay 1X Binding Buffer.
10. Add 50 µl of the resuspended MagBeads to the cells.
11. Rotate for 15 min at 4 °C.
12. Separate the MagBeads using the Magnetic Separator. Wait a few minutes for the separation to progress.
13. Carefully transfer the unbound cells from the beads to a new tube.
14. Wash the beads with 200 µl 1X Binding Buffer. Repeat Steps 12 and 13.
15. Spin the unbound cells (healthy cell population) and remove the supernatant. Keep the healthy cell population for further study.
16. The magnetic bound apoptotic cells can be used directly for apoptosis assays (e.g., caspase activity assays or others) or released from the beads using the following procedure:
    - Adding 100 µl of the Apoptotic Cell Releasing Buffer. Gently mix and incubate for 10 min at room temp. Separate the beads using the Magnetic Separator and carefully transfer the solution (containing apoptotic cells) to a new tube.
    - Repeat the releasing process again and combine the released cells together.
    - Spin down the apoptotic cells and save for further study.

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- Caspase-Mediated Apoptosis Products
- Nuclear-Mediated Apoptosis Products
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- Many Apoptosis Detection Kits, Reagents, and Antibodies
- JNK Activity Screening & Immunoassay Kits
- Histone Deacetylase Fluorometric & Colorimetric Assay Kits
- Calpain Activity Assay Kit
- Annexin V-FITC, Cy3, -Cy5, -EGFP, -PE, -Biotin Kits & Bulk Reagents
- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Cell Proliferation & Cell Viability Assay Kits
- Growth Factors, Cytokines, & Chemokines (Proteins & Antibodies)
- Quality Antibodies

FOR RESEARCH USE ONLY! Not to be used on humans.

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