ELISA PRODUCT INFORMATION & MANUAL

Apoptotic Cell Isolation Kit

NBP2-54882

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Table of Contents

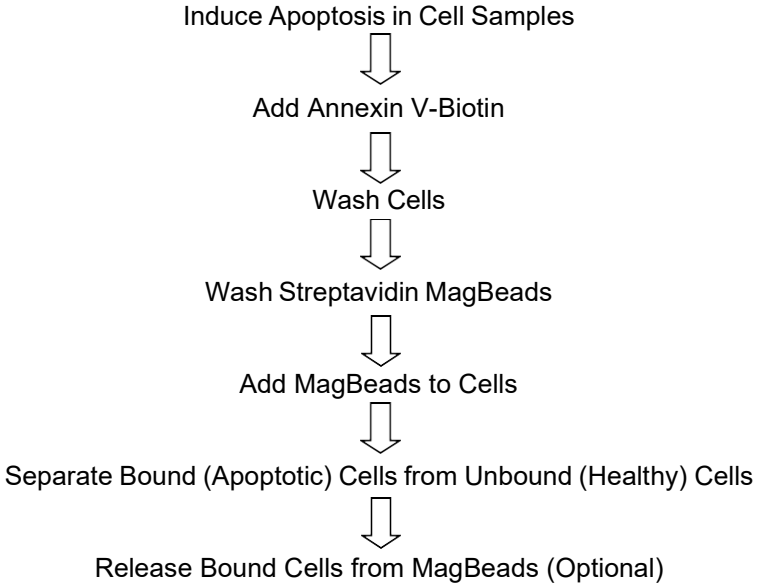
1. Overview

Novus Biologicals's 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 culture.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Annexin V-Biotin	150 µL
1X Binding Buffer	25 mL
Streptavidin MagBeads	1.5 mL
Apoptotic Cell Releasing Buffer	10 mL

* Store kit at -20°C.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Magnetic separator
- Orbital shaker

4. Assay Protocol

1. Induce apoptosis by desired method.

2. Collect 5-10 x 10^6 cells by centrifugation.

3. Re-suspend 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. Re-suspend the cells in 200 µl 1X Binding Buffer.

9. Wash the Streptavidin MagBeads with 1X Binding Buffer:

a) Transfer 50 µl/assay of the MagBeads suspension to a new tube.

b) Separate the beads by a magnetic separator and remove the solvent.

c) Add 200 µl 1X Binding Buffer to the Beads.

d) Separate the Beads again and re-suspend them in 50 µl/assay 1X Binding Buffer.

10. Add 50 µl of the re-suspended 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. Spin the unbound cells (healthy cell population) and remove the supernatant. Keep the healthy cell population for further study.

15. 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:

a) Add 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.

b) Repeat the releasing process again and combine the released cells together.

c) Spin down the apoptotic cells and save for further study.