APOPTOTIC CELL SEPARATION KIT

ProductInformation

Product Number **APO-SEP-1**
Storage Temperature 2-8°C

TECHNICAL BULLETIN

Product Description

The annexins are a group of homologous proteins that bind phospholipids in the presence of calcium. Apoptosis, or programmed cell death, is an important mechanism of most cells used to negatively select cells that are deleterious to the host. Many cells of the immune system such as thymocytes, self-reactive B and T cells undergo apoptosis as a result of the normal cell selection process. The cellular changes involved in the process include loss of cell membrane phospholipid asymmetry during the early stages of apoptosis. In living cells the phophatidylserine [PS] is transported to the inside of the lipid bilayer by the enzyme Mg-ATP dependent aminophopho-lipid translocase. However, during the onset of apoptosis, PS normally found on the internal face of the plasma membrane becomes translocated to the external face of the membrane. Thus, it becomes available for binding to annexin V and any of its conjugates in the presence of calcium ions. [1] Binding of annexin V- biotin to apoptotic cells followed by binding of the biotin to streptavidin-magnetic beads conjugates enables separation of apoptotic cells from living cells. The apoptotic cells are bound to the magnetic beads and adhere to the magnet, while living cells stay in suspension. The separated apoptotic and living cells can then be used in a variety of assays, such as determination of caspase activity.

Components

This kit includes reagents for 30 tests

A 7810 Annexin V-biotin – 1° vial (150µl).
A solution in 50 mM Tris-HCl, pH 7.5 containing 100 mM NaCl.

S 2415 Streptavidin, Magnetic beads conjugate – 2 ml
A suspension in 0.85% NaCl, 0.01 M phosphate buffered saline, pH 8.0, containing 0.1% BSA and 0.1% sodium azide.

B 9796 10X Binding Buffer – 20 ml
100 mM HEPES/NaOH, pH 7.5 containing 1.4 M NaCl and 25 mM $CaCl_2$.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution.
Consult the MSDS for information regarding hazardous and safe handling practices.

Preparation Instructions

Reagents needed but not supplied with this kit:

1. Cells to undergo apoptosis. An example is given using Jurkat E6-1 cells.
2. Apoptosis inducer. Induction may be spontaneous or induced. In the example, staurosporin [Product Code S 4400] dissolved at 100 µg/ml in DMSO is used as inducer.
3. Whole cell extract buffer: 20 mM Tris buffer, pH 7.5, containing 400 mM NaCl, 20% (v/v) glycerol, 2 mM DTT, and 1:100 Protease Inhibitor Cocktail (Product Code P 8340). In the example, this buffer is used to lyse the cells.

Reagents for determination of caspase –3 activity
In the example this activity is determined:

1. Ac-DEVD-AMC (Caspase-3 substrate,Product Code. A 1086, M.W. 675.6) – 10 mM in DMSO. Dissolve a 5 mg vial in 0.74 ml DMSO.
2. Assay buffer: 25 mM Hepes pH 7.4, 2 mM EDTA, 0.1% CHAPS, 5 mM DTT. Prepare 10 ml, keep frozen at –20°C.

Reagents supplied with this kit:
Binding buffer 1X - Dilute B 9796, (Binding buffer 10X), 1:10 with deionized water to yield 10 mM HEPES/NaOH, pH 7.5, containing 140 mM NaCl and 2.5 mM $CaCl_2$.

Equipment needed for the procedure

1. Magnetic separator for microfuge tubes –Product Code M 1167.
2. Serological centrifuge
3. Incubator at 37°C with 5% CO_2.
4. Microfuge centrifuge
5. Fluorimeter. In the example, caspase-3 activity is determined by a fluorimetric test
6. Microwell plates for fluorimeter

Storage/Stability
Store at 2-8°C.

Procedure
An example using Jurkat cells.
1. Keep non-induced cells for a zero time control.
2. Induce apoptosis in a cell suspension of Jurkat cells (e.g. by addition of staurosporin to 1 μg/ml).
3. Incubate for 2 –2.5 hr at 37°C in a 5% CO_2 atmosphere.
4. Centrifuge the induced and the control cells by centrifugation at 600 X g for 5 min at 4°C.
5. Remove the supernatant entirely by gentle suction.
6. Suspend the cells in 1X binding buffer at a concentration of 10^7 cells per 100 μl.
7. Dispense the cells in 100 μl portions in microfuge tubes .
8. Add 5 μl Annexin-V- biotin to the cells
9. Mix gently and incubate for 10-15 min on ice or at RT.
10. Centrifuge the cells for 2 min. at 600 X g.
11. Remove the buffer.
12. Suspend the cells gently in 100 μl 1X binding buffer.
13. Wash the magnetic beads with 1X binding buffer:
    - Transfer (N+1) x 50 μl beads suspension to a new tube (N =the number of tests you run).
    - Separate the beads by the magnetic separator and remove the solvent.
    - Remove the tube from the separator and add 500 μl 1X binding buffer to the beads.
    - Separate the beads again and suspend them in 1X binding buffer in the original volume
14. Add 50 μl of suspended magnetic beads to the cells
15. Rotate for 15 min at 4°C.
16. Separate the magnetic beads using the microfuge tube magnetic separator. Wait a few minutes for the process to progress.
17. Carefully transfer the unbound cells from the beads to a new tube using pipette.
18. Spin the unbound cells and remove the supernatant.
19. Add 50 μl whole cell extract buffer to each of the tubes containing beads and to each of the tubes containing the unbound cells.
20. Freeze at –80°C.
21. After a minimum of 15 min., thaw the samples.
22. Centrifuge at 12,000 rpm at 4°C for 10 minutes.
23. Transfer the supernatants to new tubes.
24. Measure caspase 3 activity.

Caspase 3 Activity assay
Reagents and equipment:
1. Prepare reaction mix: Add 5 μl of 10 mM Ac-DEVD-AMC to 3 ml of Assay buffer
2. Set the fluorimeter: exitation –380 nm, emmision – 445 nm, slit – 5

Procedure
1. Pipet 5 μl of each cell lysate into a microtiter plate well.
2. Pipet 5 μl of the whole cell extrate to a control well.
3. Add 200 μl of reaction buffer.
4. Mix by pipetting.
5. Incubate at 37°C for 20-30 min.
6. Measure fluorescence of the plate. Zero on the control well.

References
1. Van Engeland, M., et al., Cytometry, **31**, 1 (1998).

lpg 10/99