Product Information

Annexin V-FITC Apoptosis Detection Kit

APOAF

Storage Temperature 2-8 °C

Product Description

The Annexin V-FITC Apoptosis Detection kit detects apoptotic cells by flow cytometry. The annexins are a group of homologous proteins which bind phospholipids in the presence of calcium.[1,2] Annexin V-FITC is a fluorescent probe which binds to phosphatidylserine in the presence of calcium. Apoptosis, or programmed cell death, is a mechanism of cells used to negatively select cells that are deleterious to the host. The cellular changes involved in the process include loss of phospholipid asymmetry during the early stages of apoptosis. In living cells, phosphatidylserine is transported to the inside of the lipid bilayer by the Mg-ATP dependent enzyme, aminophospholipid translocase.[3] At the onset of apoptosis, phosphatidyl-serine, which is normally found on the internal part of the plasma membrane, becomes translocated to the external portion of the membrane. The phosphatidyl-serine becomes available to bind to the annexin V-FITC conjugate in the presence of calcium.

The procedure consists of the binding of annexin V-FITC to phosphatidylserine in the membrane of cells, which are beginning the apoptotic process, and the binding of propidium iodide to the cellular DNA in cells where the cell membrane has been totally compromised. Apoptosis may be either spontaneous or induced by incubating the cells with staurosporine.[4] The cells are incubated with annexin V-FITC and propidium iodide. After a 10-minute incubation period at room temperature the cells are analyzed by flow cytometry. Annexin V-FITC is detected as a green fluorescence and propidium iodide is detected as a red fluorescence.

Reagents

The reagents supplied in this kit are 0.2 μm filtered and aseptically filled.

Annexin V-FITC Conjugate (Cat. No. APOAFA) ~50 µg/mL in 50 mM Tris-HCl, pH 7.5, containing 100 mM NaCl

Propidium Iodide Solution (Cat. No. P2667)100 µg/mL in 10 mM potassium phosphate buffer, pH 7.4, containing 150 mM NaCl

10× Binding Buffer (Cat. No. B9796) 100 mM HEPES/NaOH, pH 7.5 containing 1.4 M NaCl and 25 mM $CaCl_2$

Reagents and Equipment Required

(but Not Provided)

Cat. Nos. have been given where appropriate.

- Staurosporine, Cat. No. S5921 or S6942
- DMSO, Cat. No. D8418
- Dulbecco's phosphate buffered saline (DPBS), 0.2 µm filtered, Cat. No. D8662
- 12 × 75 mm test tubes
- Cells to undergo apoptosis
- Serological centrifuge
- Incubator at 37 °C, 5% CO_2
- Flow cytometer

Precautions and Disclaimer

This product is for research use only, not for drug, household, or other uses. Annexin V is a product of human origin; handle as if capable of transmitting infectious agents. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Allow all kit components to reach room temperature before use.

Prepare 1× Binding Buffer by diluting 1 mL of the 10× Binding Buffer with 9 mL of deionized water.

Dissolve the staurosporine in DMSO to a concentration of 100 µg/mL or use Staurosporine solution (S6942).

Storage/Stability

The kit ships on wet ice and storage at 2–8 °C is recommended.

Procedure

This procedure describes the induction of apoptosis in the Jurkat cell line, followed by measurement of phosphatidylserine. Perform the experiment using aseptic technique.

1. Induce apoptosis in a 1 x 10^6 cells/mL suspension of Jurkat cells by the addition of 1 µg/mL staurosporine.
2. Establish a control of non-induced Jurkat cells at 1 x 10^6 cells/mL for a zero-time data point.
3. Incubate both Jurkat cell cultures for 1–2 hours in a 37 °C, 5% CO_2 incubator.
4. Wash the cells twice with DPBS.
5. Resuspend the cells in 1× Binding Buffer at a concentration of ~1 x 10^6 cells/mL.
6. Add 500 µL of the apoptotic cell suspension to a plastic 12 × 75 mm test tube.
7. Add 500 µL of the non-induced cell suspension to a second plastic 12 × 75 mm test tube.
8. Add 5 µL of Annexin V-FITC Conjugate and 10 µL of Propidium Iodide Solution to each cell suspension.
9. Incubate the tubes at room temperature for exactly 10 minutes and protect from light.
10. Determine the fluorescence of the cells immediately with a flow cytometer. Cells, which are early in the apoptotic process, will stain with the Annexin V-FITC Conjugate alone. Live cells will show no staining by either the Propidium Iodide Solution or Annexin V-FITC Conjugate. Necrotic cells will be stained by both the Propidium Iodide Solution and Annexin V-FITC Conjugate.

Related Products

Annexin V from human placenta (Cat. No. A9460)

Annexin V-FITC Conjugate (Cat. No. APOAFA)

References

1. Pigault, C., et al., J. Mol. Biol., 236: 199 (1994).
2. Trotter, P., et al., Biochem. J., 308: 591 (1995).
3. Kuypers, F. A., et al., Blood, 87: 1179 (1996).
4. Martin, S. J., et al., J. Exp. Med., 182: 1545 (1995).

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