

Annexin V-FITC/PI Apoptosis Assay kit

Packing specification

Product number: PI0020, PI0050, PI0100

Specifications: 20T, 50T, 100T

Storage conditions

Stored at 4°C, valid for one year

Reagents:

Reagents	20 assays	50 assays	100 assays	Storage
Annexin V-FITC product code:AF001	100 µl	250 µl	500 µl	4°C in the dark
Propidium Iodide, PI product code:PP002	200 µl	500 µl	1000 µl	4°C in the dark
Binding Buffer (4×) product code:BB002	4 ml	10 ml	20 ml	4°C

Precautions and Disclaimer

This product is for R&D 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.

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.

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 1×10^6 cells/ml suspension of Jurkat cells by the addition of 1 µg/ml staurosporine.
2. Establish a control of non-induced Jurkat cells at 1×10^6 cells/ml for a zero time data point.
3. Incubate both Jurkat cell cultures for 1–2 hours in a 37 °C, 5% CO₂ incubator.
4. Wash the cells twice with DPBS.
5. Resuspend the cells in 1× Binding Buffer at a concentration of $\sim 1 \times 10^6$ 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.

It can only be used for scientific research. It is forbidden to use it for human, animal or other purposes.