ELISA PRODUCT INFORMATION & MANUAL

Annexin V Apoptosis [FITC]

NBP2-29373

Apoptosis Detection Kit.
For research use only. Not for diagnostic or therapeutic procedures.

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ANNEXIN V-FITC STAINING PROTOCOL

Components of the Kit:

Vial Description	Contents	Storage Conditions
Largest Bottle-Brown Cap	10x PBS Solution	2-8C
Smaller Bottle-Blue Cap	10X Binding Buffer	2-8C
Smallest Bottle-Blue Cap	Compensation Control Cells	2-8C
Small Vial-Brown Cap	Propidium Iodide Solution	2-8C
Small Vial-Green capped	Annexin V-FITC	2-8C

Reagent Preparation:

The Annexin V-FITC, Propidum Iodide (PI) Solution and the Compensation Control cells are provided ready to use. There are enough reagents to perform 100 assays and enough Control Cells for 10 reactions. The 10X Annexin V Binding Buffer and 10X PBS Solution need to be diluted prior to use. To prepare a 1X, working dilution, of each of these, dilute 1 part buffer and PBS to 9 parts distilled H_2O (dH_2O).

Staining:

1. Determine the number of tubes that need to be run and prepare sufficient amounts of both the 1X PBS and Binding Buffer as described above.
2. Label 3 tubes according to the table below and additional tubes for experimental samples as needed.

Tube #	Cells	Stain
1	Stabilized Control Cells	---
2	Stabilized Control Cells	5µl Annexin V-FITC
3	Stabilized Control Cells	5µl PI Solution
4	Un-induced Experiment Control	5µl Annexin V-FITC + 5µl PI
5	Apoptosis Induced Experimental Sample	5µl Annexin V-FITC + 5µl PI

3. Gently mix the Control Cells by swirling the bottle to ensure an even distribution of the cells. Remove 100µl of cell suspension from the vial and transfer to each of the appropriately labeled tubes according to the above table.
4. Add 1ml of cold 1X PBS to each tube and spin down at 300 x g for 5 minutes.
5. Carefully remove supernatant as to not disrupt the cell pellet.
6. Repeat step #4.
7. After carefully removing the supernatant, re-suspend the cell pellet in 100µl of 1X binding buffer by "flicking" the tube. DO NOT VORTEX.
8. Add staining reagents according to the table above. Gently mix the cells by "swirling" the tube.
9. Incubate for 20 minutes at room temperature in the dark.
10. Add 400µl of 1X binding buffer to each tube.
11. Analyze on the flow cytometer within 1 hour.