Annexin Binding Buffer(5×），for flow Cytometry

Catalog Number： R21912

Amount： 50ml/100ml

Product description：

Annexins are a family of calcium-dependent phospholipid-binding proteins that preferentially bind phosphatidyl serine (PS). Under normal physiological conditions, PS is predominantly located in the inner leaflet of the plasma membrane. Upon initiation of apoptosis, PS loses its asymmetric distribution across the phospholipid bilayer and is translocated to the extracellular membrane leaflet, marking cells as targets for phagocytosis. Once on the outer surface of the membrane, PS can be detected by fluorophore-labeled annexin V in a calcium-dependent manner.

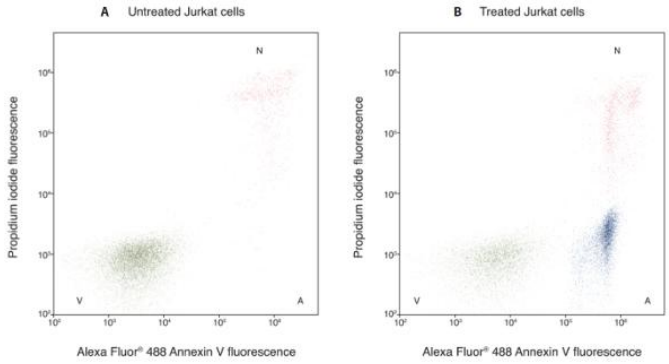

Figure 1　10μM camptothecin treated Jurkat cells and untreated control

Jurkat cells (human T-cell lymphocytes) treated with 10μM camptothecin for 4 hours (panel B) or untreated control (panel A). Alexa Fluor™ 488 Annexin V and Propidium Iodide Dead Cell Stain were used with the Annexin Binding Buffer. Cells were stained and analyzed by flow cytometry using 488-nm excitation on the Attune™ NxT Acoustic Focusing Cytometer, 530/30-nm and 575/24-nm bandpass filters, and collected by means of a standard 100μL/minute collection rate. Note that the camptothecin treated cells have a higher percentage of apoptotic cells (panel B) than the basal level of apoptosis seen in the control cells (panel A). A = apoptotic cells, V = viable cells, N = necrotic cells.

Contents and storage：

Product	Composition	Amount	Storage
Annexin Binding Buffer(5×），for flow Cytometry	50 mM HEPES, 700 mM NaCl, 12.5 mM CaCl2, pH 7.4	50ml/100ml	2~8°C

Required materials not supplied：

1. Deionized water
2. Cold phosphate-buffered saline (PBS)

Before you begin：

Prepare 1×　Annexin Binding Buffer by diluting Annexin Binding Buffer (5×) with deionized water. Store the diluted buffer at 2–8°C. The final 1×　concentration of Annexin Binding Buffer is 10 mM HEPES, 140 mM NaCl, 25 mM CaCl2, pH 7.4.

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For example, for 10 assays, add 1mL of Annexin Binding Buffer (5×) to 4mL of deionized water.

Label apoptotic cells for flow cytometry：

Note: This assay is optimized for use with Jurkat cells treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types.

1. Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.
2. Harvest the cells after the incubation period, then wash in cold PBS.
3. Centrifuge the washed cells from step 2 again, then discard the supernatant.
4. Resuspend the cells in 1× Annexin Binding Buffer.
5. Count the cells, then adjust the cell density to ～1× 10^6 cells/mL with 1× Annexin Binding Buffer. Prepare a sufficient volume to use 100 µL per assay.
6. Add the appropriate amount of annexin V conjugate and dead cell dye to each 100 µL of cell suspension.
7. Incubate the cells for 15 minutes at room temperature.
8. Add 400 µL of 1× Annexin Binding Buffer, mix gently, then place the samples on ice.
9. Immediately, analyze the stained cells by flow cytometry.