Annexin Binding Buffer (5X), for flow cytometry

Catalog Number V13246

Pub. No. MAN0003219 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

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.

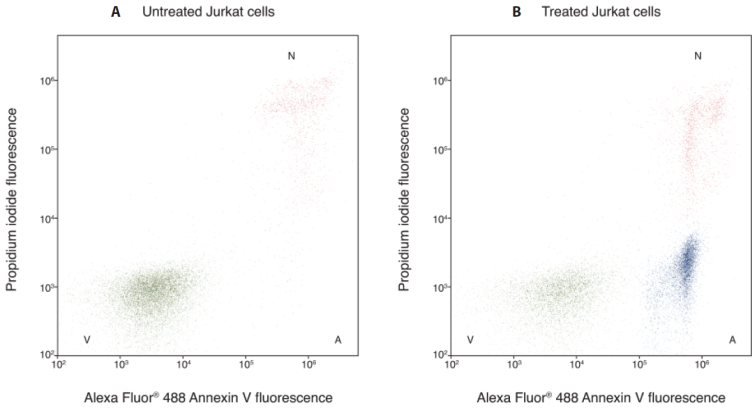

Figure 1 10 µM camptothecin treated Jurkat cells and untreated control
Jurkat cells (human T-cell lymphocytes) treated with 10 µM camptothecin for 4 hours (panel B) or untreated control (panel A). Alexa Fluor™ 488 Annexin V and Propidium Iodide Dead Cell Stain were used with the Annexin Binding Buffer. 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 100 µL/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	Cat. No.	Composition	Amount[1]	Storage[2]
Annexin Binding Buffer (5X), for flow cytometry	V13246	50 mM HEPES, 700 mM NaCl, 12.5 mM $CaCl_2$, pH 7.4	50 mL	2 to 6°C Do not freeze.

[1] Sufficient material is supplied for 500 assays based on the protocol described in this user guide.
[2] When stored as directed, this product is stable for 1 year from the date of receipt.

Required materials not supplied

- Deionized water
- Cold phosphate-buffered saline (PBS)

Before you begin

Prepare 1X Annexin Binding Buffer by diluting Annexin Binding Buffer (5X) with deionized water. Store the diluted buffer at 2–8°C. The final 1X concentration of Annexin Binding Buffer is 10 mM HEPES, 140 mM NaCl, 25 mM $CaCl_2$, pH 7.4.

For example, for 10 assays, add 1 mL of Annexin Binding Buffer (5X) to 4 mL 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 1X Annexin Binding Buffer.
5. Count the cells, then adjust the cell density to ~1 x 10^6 cells/mL with 1X 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 1X Annexin Binding Buffer, mix gently, then place the samples on ice.
9. Immediately, analyze the stained cells by flow cytometry.

Related products

For more information on other products for apoptosis research, visit **thermofisher.com/apoptosis**.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0003219

Revision	Date	Description
A.0	3 May 2022	The content and document layout were updated. This document supercedes Rev 1.0, revision date September 2010.