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Annexin V Binding Buffer 10X

Reference	Size
BB10X-50ML	50 ml

PRODUCT DESCRIPTION

Exposure of PS on the external surface of the cell membrane has been reported in apoptotic cells, this occurs in the early phases of apoptotic cell dearth during which the cell membrane remains intact. In leukocyte apoptosis, PS on the outer surface of the cell marks the cell for recognition and phagocytosis by macrophages. Under defined salt and calcium conditions, annexin V can identify apoptotic cells by binding to PS exposed on the outer leaflet.

This product is used to facilitate the binding of annexin V to phosphatidylserine for use in apoptosis assays and is supplied as a 10X solution. It should be diluted to 1X in deionized water before using.
The pH of the 1X solution should fall within the range of pH 7.3-7.4. Adjust the pH if necessary. Warm the 1X solution to room temperature prior to use.
Samples must be prepared as single cell suspension in an appropriate tube.

Recommended usage: Immunostep's Annexin V binding buffer, is intended for flow cytometric labelling of apoptotic cells with Annexin V reagents.

Presentation: liquid

Storage Instruction: store Annexin V binding buffer between 2°C and 8°C.

Reagent provided: 50 ml of 10X concentrate 0,1M Hepes/NaOH (pH 7,4) 1,4 M NaCl, 25 mM $CaCl_2$.

Recommendation and warnings: This product contains sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded. These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop.
For professional use only.
Do not use after expiration date stamped on vial.

Staining cells protocol with Annexin-FITC. Flow Cytometry

1. Prepare 1X Annexin V Binding Buffer
2. Induce apoptosis in cells using the desired method. A negative control should be prepared by untreated cells, that is used to define the basal level of apoptotic and necrotic or dead cells.
3. Harvest the cells after the apoptosis induction and wash in temperate phosphate-buffered saline (PBS).
4. Wash cells twice with temperate PBS and resuspend cells in 1 X Annexin-binding buffer at a concentration 1 x 10^6 cells/ml.
5. Add 5 µl of the Annexin V and 5 µl of PI/7-AAD, to each 100µl of cell suspension.
6. Incubate the cells at room temperature for 15 minutes at room temperature (25°C) in the dark.
7. After incubation period, add 400 µl of 1X Annexin-binding buffer. Analyze by flow cytometry within one hour.

Example Protocol for Annexin V expression in apoptotic peripheral blood lymphocytes

1. MN-Cells (Mononuclear cells) have been separated by Ficoll, from peripheral blood.
2. Apoptosis induction in leukocytes incubating 6 hours with H_2O_2 200µM.
3. 1 million cells have been harvested after the apoptosis induction. The supernatant was removed by centrifugation.
4. Added 100µL of PBS and 20µL of Conjugated CD19 antibody and incubated 15 min.
5. Wash the cells once with temperate PBS and resuspend in 0,5 ml of 1 X Annexin-binding buffer.
6. Added 5 µl of the Annexin V and 5 µl of PI/7-AAD, to cell suspension.
7. Incubate the cells for 15 minutes at room temperature, and analyze by flow cytometry.

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services. (tech@immunostep.com)

Please, refer to **http://immunostep.com/content/31-support** for technical information.

WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

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