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Annexin V Binding Buffer 10X

Product Information.

Catalog Ref: BB10X-50ML

Size: 50 ml (X10)

Introduction.

Apoptosis is a regulated process of cell death that occurs during embryonic development as well as maintenance of tissue homeostasis. Inappropriately regulated apoptosis is implicated in different disease states, such as neurodegeneration disease and cancer. The apoptosis program is characterized by morphologic features, including loos of plasma membrane asymmetry and attachment, condensation off the cytoplasm and nucleus, and compaction and fragmentation of the nuclear chromatin. In normal viable cells, phospholipids are asymmetrically distributed between inner and outer leaflets of the plasma membrane whit phosphatidylcholine and sphingomyelin exposed on the external leaflet of the lipid bilayer, and phosphatidylserine predominantly observed on the inner surface facing the cytosol.

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. The human vascular anticoagulant, annexin V, is a 35-36 kDa Ca^{2+} dependent phospholipids binding protein that has a high affinity for PS, and shows minimal binding to phosphatidylcholine and sphingomyeline. Changes in PS asymmetry, which is analyzed by measuring annexin V binding to the cell membrane, were detected before morphological changes associated with apoptosis have occurred and before membrane integrity has been lost. Annexin V labelled with FITC (green fluorescence) can identify and quantitate apoptotic cells on a single-cell basis by flow cytometry. Staining cells simultaneously with Annexin V –FITC and the non-vital dye propidium iodide (red fluorescence) allows (bivariate analysis) the discrimination of intact cells (Annexin V-FITC negative, PI negative), early apoptotic (Annexin V-FITC positive, PI negative) and late apoptotic or necrotic cells (Annexin V-FITC positive, PI positive).

Materials.

Annexin V Binding Buffer, 10 X, 50 ml. 0,1M Hepes/NaOH (pH 7,4) 1,4 M NaCl, 25 mM $CaCl_2$.

Storage.

Store in the dark at 2-8 °C. Do not use after expiration date stamped on vial. 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
DO NOT FREEZE. Protect the fluorescent conjugates from the light.

Staining cells protocol with Annexin-FITC. Flow Cytometry

1. Prepare Annexin V Binding Buffer: 10 mM Hepes/NaOH (pH 7,4) 140 mM NaCl, 2,5 mM $CaCl_2$. .
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-FITC and 5 µl of PI, to each 100µl of cell suspension.
6. Incubate the cells at room temperature for 15 minutes at room temperature (25ºC) 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 100uL of PBS and 20uL of the CD19 APC(Ref. CD19APCA3-B1 - IMMUNOSTEP) and incubated 15 min.
5. The cells was washed once with temperate PBS and the cells was resuspend in 1 X Annexin-binding buffer 0,5 ml.
6. Added 5 µl of the Annexin V-FITC and 5 µl of PI, to cell suspension.
7. The cells was incubated for 15 minutes at room temperature, and have been analyzed by flow cytometry

Controls to set up flow cytometer compensation and quadrants.

1. Unstained cells.

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2. Cells stained with Annexin V- FITC alone (no PI).
3. Cells stained with PI alone (no Annexin V-FITC).

Troubleshooting.

a. Absence of Annexin V-FITC fluorescence: apoptosis was not induced in the cells.

b. Elevated Annexin V-FITC and/or PI stainability: apoptosis is an ongoing process so that cells stained with Annexin V should not be kept for prolonged times before measurement. Cells which still maintain membrane integrity for longer incubation times may becomes positive for PI since this dye will enter intact cells although very slowly. Analyze cells as soon as are stained and you can add PI solution just before the analysis.

c. Adherents cells may be released from their substrate by using trypsin. Trypsinized cells can be affected in the integrity of the plasma membrane [5]. On adherent cells a good idea is to remove supernatant with floating cells and replace media before adding drugs, or remove culture medium from cells, and immerse slide into cold (2-8 °C) 1X PBS.

d. Target cells that have been stained with Conventional annexin V-FITC/PI kit and then fixed with 1% PFA or methanol, they can give a signal quenching. In this case, probably, you have used an excessive dilution of the Binding Buffer, the fixation method can be optimized by using the $CaCl_2$ in the 10X Binding Buffer to 25 mM (2.5 mM final concentration).

References.

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