Annexin V Apoptosis Detection Kit

Ready-to-use

Ref.: AnxF100PI Annexin-V-FITC / Propidium iodide

Ref.: AnxA100PI Annexin-V-APC / Propidium iodide

Ref.: AnxB100PI Annexin-V-Biotin / Propidium iodide

MabTag GmbH

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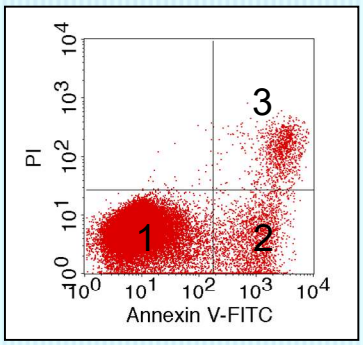
Background

MabTag's Annexin V Apoptosis Detection Kit is designed to rapidly analyse different stages of apoptosis and cell death in single cell suspensions.
Annexin V binds specifically to phosphatidylserine (PS) which is located to the inner side of the cytoplasmic membrane in intact cells. But after initiation of apoptosis PS becomes rapidly translocated to the outer side of the cytoplasmic membrane where it is thought to play an important role in macrophage recognition, thus allowing the apoptotic cells to become rapidly phagocytosed.
The binding of annexin V to PS is Ca^{2+}-dependent and therefore, a specific Ca^{2+}-containing reaction buffer is needed during the binding process. At low PS concentrations a binding ratio of eight annexin V molecules to one PS has been reported making annexin V-conjugates ideal for identifying membrane changes associated with early apoptotic events.
In late apoptosis/necrosis cells loose their membrane integrity which allows further annexin V binding to PS located to the inner cell membrane. Therefore, MabTag's Annexin V Apoptosis Detection Kit contains a viability dye such as propidium iodide (PI). This DNA-intercalating dye can only pass membranes which lost their integrity and therefore, allow the discrimination of early apoptotic cells (Annexin V^+/PI^-) from late apoptotic/necrotic cells (Annexin V^+/PI^+).



Cells were treated with UV for 30 minutes. After an incubation of 4 hours they were subsequently stained according to the procedure described below.
1. viable cells (double-negative)
2. early apoptotic cells (annexin V-positive/PI-negative)
3. late apoptotic/necrotic cells (double-positive)

Reagents provided

500 µl Annexin V FITC / APC / Biotin conjugate; (with 1% BSA / 0.1% NaN_3 as preservative)
500 µl propidium iodide [20 µg/ml]
10 ml Annexin V binding buffer (10fold concentrate)

Characteristics of Fluorochromes

Fluorochrom	Absorption peak (nm)	Emission peak (nm)	Excitation at (nm)
FITC	495	519	488
APC	650	660	633
Propidium iodide	536	617	488

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Storage

Upon arrival store all reagents in the dark at 2 - 8 °C.
If stored properly all reagents will be stable for 12 months.

Materials/Reagents required

Flow cytometer or fluorescence microscope
Microcentrifuge
Microcentrifuge tubes
Adjustable pipettors
Pipette tips
Gloves
Deionized or destilled water
PBS and/or culture medium

Precautions and limitations

For research use only. Not for use in diagnostic procedures.
Propidium iodide and sodium azide (NaN_3) are toxic. Wear gloves, lab coat, and eye protection when using it. The physical, chemical, and toxicological properties of this kit's components may not yet have been fully investigated. Therefore, MabTag recommends the use of gloves, lab coat, and eye protection when using these chemicals. MabTag assumes no liability for damage resulting from handling or contact with these chemical reagents.
Annexin V conjugates and propidium iodide are light sensitive and should be kept in the dark during storage and staining procedure.

Staining procedure

1. Dilute an appropriate amount of 10fold Annexin V binding buffer with deionized or distilled water in 1:10 ratio (mix 1 part 10x buffer with 9 parts water)
2. Wash cells with culture medium or PBS
3. Resuspend 10^4 – 10^6 cells in 90 µl of diluted (1 fold) Annexin V binding buffer.
4. Add 5 µl of Annexin V conjugate and 5 µl of propidium iodide solution.
5. Incubate for 20 minutes in the dark.
6. Add 400 µl of Annexin V binding buffer (1fold).
7. Centrifuge at 400 x g for 5 minutes.
8. Resuspend cells in 200 µl Annexin V binding buffer (1fold) and analyze sample by flow cytometry or fluorescence microscopy.

Reference

Pathogen-induced ubiquitin-editing enzyme A20 bifunctionally shuts off NF-κB and caspase-8-dependent apoptotic cell death.
Lim MCC, Maubach G, Sokolova O, Feige MH, Diezko R, Buchbinder J, Backert S, Schlüter D, Lavrik IN, Naumann M.
Cell Death Differ. 2017 Sep;24(9):1621-1631.

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