

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

v3.2018

Reagents provided

500 µl Annexin-V FITC / APC / Biotin conjugate; (with 1% BSA / 0.1% NaN₃ as preservative) **5µl = 1 test**

500 µl propidium iodide [20 µg/ml] **5µl = 1 test**

10 ml Annexin-V binding buffer (10x concentrate)

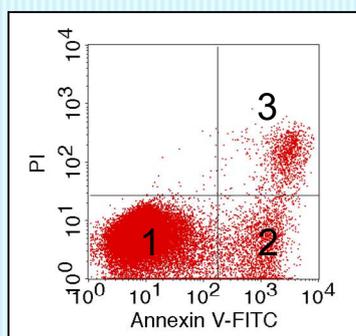
Background

MabTag's Annexin-V Apoptosis Detection Kit is designed for fast analysis of 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 Ca²⁺-dependent and therefore, a specific Ca²⁺-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 lose 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)

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

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 distilled water
PBS and/or culture medium

Staining procedure

1. Dilute an appropriate amount of 10x Annexin-V binding buffer (e.g. MabTag's Ref. AnxBuff) with deionized or distilled water in 1:10 ratio (= 1x buffer)
2. Wash cells with culture medium or PBS
3. Resuspend cells (typical $10^4 - 10^6$ cells) in 90 μ l of diluted (1x) Annexin-V binding buffer.
4. Add 5 μ l of Annexin-V conjugate (e.g. MabTag's Ref. AnxF100) and 5 μ l of propidium iodide solution.
5. Incubate 20 minutes in the dark.
6. Add 400 μ l of Annexin-V binding buffer (1x).
7. Centrifuge at 400 x g for 5 minutes.
8. Resuspend cells in 1x Annexin-V binding buffer (typical $\pm 500\mu$ l) and analyze sample by flow cytometry

Reference

Pathogen-induced ubiquitin-editing enzyme A20 bifunctionally shuts off NF- κ B 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.

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.

For research use only. Not for use in diagnostic procedures.