<u>Annexin-V-Biotin</u>

Ref.: <u>AnxB100</u>

Label: Biotin

Application: Flow cytometry, no others tested

Buffer: 0.1 % BSA in PBS. Preservative: 0.09 % w/v sodium azide.

Volume: 0,5 ml (= 100 tests)

Preparation: ready to use; 5µl = 1 test

Storage: Upon arrival store at 2 - 8 °C.

If stored properly reagent will be stable for 12 months.

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

Background

Fluorochrom-conjugated recombinant chicken Annexin-V for the detection of phosphatidylserine (PS) exposed in the membrane of apoptotic cells. There is 85 % homology of recombinant chicken Annexin-V to the human Annexin-V and a 100 % identity in the phosphatidylserine binding sites. Annexin-V binding to PS is Ca⁺⁺dependent. Apoptosis and necrosis are the two main forms of cell death. Apoptosis is mostly a physiological process and plays an essential role in the development and homeostasis of all multi-cellular organisms. Apoptosis can be induced by several stimuli like UV- and γ -irradiation or DNA damaging substances. Apoptotic cells change the structure of their membrane, which leads to the exposure of PS on the membrane surface. Annexins are ubiquitous homologous proteins that bind phospholipids in the presence of calcium. Since the redistribution of PS from the internal to the external membrane surface represents an early indicator of apoptosis, Annexin-V and its conjugates can be used for the detection of apoptosis because they interact strongly and specifically with exposed PS. Detection of apoptotic cells with Annexin-V can be achieved earlier than analysis of apoptotis by DNA-based assays.

Staining procedure

1. Prepare an appropriate Ca⁺⁺-containing Annexin-V binding buffer.

By using MabTag's 10x Annexin-V binding buffer (*Ref: AnxBuff*) – dilute an appropriate amount of 10x Annexin-V binding buffer with distilled water in 1:10 ratio

- (e.g. mix 1ml 10x buffer with 9ml water = 10ml 1x buffer)
- 2. Wash cells with culture medium or PBS
- 3. Resuspend cells (typical $10^4 10^6$ cells) in 90 µl of diluted (1x) Annexin-V binding buffer.
- 4. Add 5 μl of Annexin-V conjugate (*e.g. MabTag's Ref. AnxF100*) and 5 μl of propidium iodide solution.
- 5. Incubate 20 minutes in the dark.
- 6. Add 400 µl 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 ±500µl) and analyze sample by flow cytometry

Reference

Effect of Lysosomotropic Polyamineoxidase Inhibitor MDL-72527 on Platelet Activation. Liu G, Cao H, Liu G, Heinzmann D, Chen H, Umbach AT, Gawaz M, Lang F. Cell Physiol Biochem. 2016;38(5):1695-702.

Warning:

Sodium azide is harmful if swallowed (R22). Keep out of reach of children (S2). Keep away from food, drink and animal feedingstuff (S13). Wear suitable protective clothing (S36). If swallowed, seek medical advice immediately and show this container or label (S46). Contact with acids liberates very toxic gas (R32). Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions can develop. This material is offered for research only. Not for use in human. For in vitro use only. MabTag will not be held responsible for patent infringement or other violations that may occur with the use of our products.

MabTag

GmbH

v3.2018