Lectin-Narcissus-Pseudonarcissus Apoptosis

FITC - conjugated

Cat-No: **31490023** 500µl for 100 tests

please note: store at –20°C

(FITC)-conjugated lectin from *Narcissus Pseudonarcissus* (NPn) for the detection of alpha-D-mannosyl residues of glycoconjugates after short term acid treatment.

The NPn method is the first one for the detection of apoptosis, which recognizes changes in structures of glycoconjugates which are accessible after acid treatment. Besides the binding of Annexin V to PS, the method is suitable for the detection of structural changes of membranes from cells undergoing apoptosis. The differentiation between apoptotic and necrotic cells can be performed by staining with propidium iodide (PI) in a separate measurement. Double-staining of lectin and PI is not recommended since the acid treatment can lead to false PI positive cells.

Buffer/Additives/Preservative: Each vial contains fluorescein conjugated lectin with 0.1 % BSA in PBS. Preservative: 0.09 % w/v sodium azide.

Application: After short term acid treatment, alpha-D-mannosyl residues of glycoconjugates become accessible for NPn binding on apoptotic as well as on necrotic cells. Viable cells do not show these changes in the structure of their membranes. Therefore, a characterisation of cells in early stages of apoptosis is possible. Necrotic cells should be detected with conventional PI staining in a second sample.

Staining procedure for flow cytometry and fluorescence microscopy
Take cells in 195 µl culture medium with 5 % BSA and add 5 µl FITC-conjugated lectin from NPn, incubate at 4°C for 30 minutes, Vortex sample for 10 seconds.
Add 300 µl of 1 % (v/v) acetic acid and vortex sample for 10 seconds.
Add 150 µl of a solution containing 2.25 % (w/w) Sodium-hydrogen-carbonate plus 1.42 % (w/w) Di-sodium-carbonate and vortex sample for 10 seconds.
Add 50 µl of 4 % (w/w) Paraformaldehyde in isotonic buffer and store sample at 4°C until measurement.
(Efficient working and compliance of the time period for vortexing is necessary)
To discriminate necrotic from apoptotic cells, a separate sample of cells should be stained with propidium iodide.

References: Heyder P, Gaipl US, Beyer TD, Voll RE, Kern PM, Stach C, Kalden JR, Herrmann M.
Early detection of apoptosis by staining of acid-treated apoptotic cells with FITC-labeled lectin from Narcissus pseudonarcissus. Cytometry. 2003 Oct;55A(2):86-93.

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.

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