For Research Use Only.
Not for use in diagnostic procedures.

Annexin V-Biotin

Code No.	Quantity
4695-100	1 mL (100 tests)

BACKGROUND: Apoptosis, a term that describes regulated cell death, is fundamental feature of many processes including normal development, homeostasis, and disease. Early during the process of apoptosis, cells lose their phospholipid membrane asymmetry and expose phosphatidylserine (PS) at the cell surface. This process can be monitored by using Annexin V which is a Ca^{2+}-dependent, phospholipid-binding protein with high affinity for PS, and is useful for identifying apoptotic cells with exposed PS. Translocation of PS to the external cell surface is not unique to apoptosis, but occurs also during cell necrosis. The difference between these two forms of cell death is that during the initial stages of apoptosis the cell membrane remains intact, while at the very moment that necrosis occurs the cell membrane looses its integrity and becomes leaky. Therefore, necrotic cells easily stained with Propidium Iodide (PI) as well as Annexin V, whereas Apoptotic cells stained only with Annexin V.

SOURCE: Recombinant annexin V protein produced in *E. coli.*

FORMULATION: Biotinylated annexin V solution is bufferlyzed with PBS (containing 0.09% NaN_3 and 1 mg/mL BSA), pH 7.2.
*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE: This product is stable for 3 years from the date of manufacture when stored at 4°C.

REACTIVITY: Annexin V-Biotin binds PS in a Ca^{2+}-dependent manner.

APPLICATION:
Flow cytometry; Ready for use

Detailed procedure is provided in the following **PROTOCOL**.

REFERENCES:
1) Taguchi T., *et al.*, *J Immunol.*, **170**, 252-260 (2003)
2) Suzuki T., *et al.*, *J Immunol.*, **166**, 5567-5577 (2001)

RELATED PRODUCTS:
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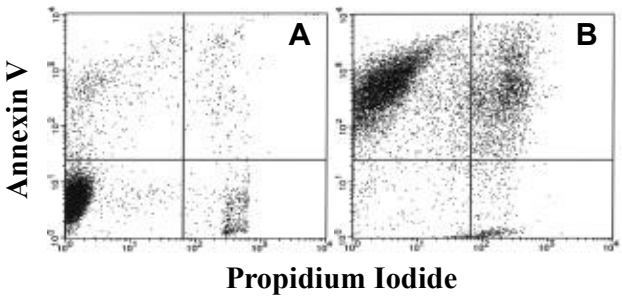

Detection of apoptotic cells by double staining with Annexin V-Biotin (code no. 4695-100) and propidium iodide. FITC labeled streptavidin was used for fluorochrome labeling.
A; Jurkat cells, non-treatment
B; Jurkat cells treated with anti-Fas (CH-11) for 4 hr.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOL:
Flow cytometry
Since apoptosis is a very rapid and dynamic process, we recommend that you perform analysis immediately after staining.

1) Induce apoptosis by desired method.
2) Wash the cells (2×10^5 cells) to be used for staining once with PBS.*
*For adherent cells, trypsinize and gently wash cells once with serum-containing media followed by wash once with PBS.
3) Resuspend the cells in 90 µL of Binding Buffer**.
4) Add 10 µL of Annexin V-Biotin and mix well, then incubate for 15 min. at room temperature (20~25°C) in the dark.
5) Wash the cells once with 1 mL of Binding buffer.
6) Add 30 µL of fluorochrome labeled streptavidin. Mix well and incubate for 30 min. at room temperature (20~25°C) in the dark.
7) Wash the cells once with 1 mL of Binding buffer.
8) Resuspend the cells with 500 µL of Binding buffer and analyze by a flow cytometer.

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Any other secondary labeling of the cells with monoclonal antibody or DNA dye is possible for further cellular characterization.

** 5 fold concentrated Binding Buffer is available as shown in **RELATED PRODUCTS**.