ApoDetect™ Annexin V-FITC Kit

Catalog Number 33-1200

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Annexins are a family of calcium-dependent phospholipid-binding proteins that preferentially bind phosphatidylserine (PS). Under normal physiologic conditions, PS is predominantly located in the inner leaflet of the plasma membrane. Upon initiation of apoptosis, PS loses its asymmetric distribution across the phospholipid bilayer and is translocated to the extracellular membrane leaflet marking cells as targets of phagocytosis. Once on the outer surface of the membrane, PS can be detected by fluorescently labeled Annexin V in acalcium-dependent manner.

In early-stage apoptosis, the plasma membrane excludes viability dyes such as propidium iodide (PI), or LIVE/DEAD™ Fixable Viability Dyes. These cells will stain with Annexin V but not a viability dye, thus distinguishing cells in early apoptosis. However, in late stage apoptosis, the cell membrane loses integrity thereby allowing Annexin V to also access PS in the interior of the cell. A viability dye can be used to resolve these late-stage apoptotic cells.

Contents and storage

Component	Amount[1]	Composition	Storage
Annexin V-FITC[2]	0.5 mL	Solution contains 50 mM Tris, 100 mM NaCl, 1% BSA, 0.01% sodium azide, pH 7.4.	2 to 8°C After opening, aliquot and freeze at –20°C
2X Binding buffer	16 mL	20 mM Hepes/NaOH, pH 7.4, 280 mM NaCl, 5 mM CaCl	2 to 8°C
Propidium iodide	0.5 mL	20 µg/mL	2 to 8°C

[1] Sufficient for 50 assays.

[2] Source: *E. coli*; Molecular weight: 35.8 kDa; Purity: >98% as demonstrated by SDS gel electrophoresis and reverse-phase HPLC.

Stain cells with Annexin V-FITC

1. Aliquot approximately 10^5 to 10^6 cells per sample.
2. Centrifuge cells for 5 minutes at 400 x *g*. Decant supernatant.
3. Wash cells in ice cold PBS, pH 7.4. Gently resuspend pellet.
4. Centrifuge cells for 5 minutes at 400 x *g*. Decant supernatant.
5. Dilute 2X binding buffer with equal amount of deionized water to a final 1X concentration (10 mM Hepes/NaOH, pH, 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$).
6. Resuspend cells in 190 µL of 1X binding buffer.
7. Add 10 µL of Annexin V-FITC to the cell suspension. Mix gently.
8. Incubate 10 minutes at room temperature.

9. Wash cells once with 1X binding buffer. Centrifuge cells for 5 minutes at 400 x *g*. Decant supernatant and resuspend in 190 µL of 1X binding buffer.

10. Add 10 µL of 20 µg/mL propidium iodide stock solution.

11. Analyze cells by flow cytometry or fluorescence microscopy.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0003469

Revision	Date	Description
A.0	5 April 2022	The format and content were updated. This document supercedes Rev 1.01, revision date January 2012.