Annexin V-FITC Apoptosis Detection Kit

Ordering info

TBK0508, 20 reactions

TBK0509, 100 reactions

Description

Annexin V-FITC Apoptosis Detection Kit is an effective kit designed to dual detection of apoptotic stage: collapse of asymmetrical distribution of phosphatidylserine (PS) and destabilization of membrane integrity. Annexin V binds with high affinity to PS (Kd ~ $5x10^{-2}$) in Ca^{2+} presence and this binding is detected by its fluorochrome conjugated. Propidium iodide only can enter into late apoptotic or necrotic cells, to bind to DNA.

Features

- Earlier Apoptosis Detection than DNA-based assays.
- Dual detection of early- and late-stage cell apoptosis.
- Versatile, suitable for adhesion and suspension cells.

Applications

- Determination of early-stage cell apoptosis.
- Determination of late-stage cell apoptosis.
- Differentiation of apoptosis and necrosis cells.

Kit Components

Components	TBK0508	TBK0509
Annexin V-FITC[a]	0.1 mL	0.5 mL
Annexin Binding Buffer 10x[b]	10 mL	50 mL
Propidium Iodide[a]	0.3 mL	1 mL

Order Info Kit Components: *Annexin-V-FITC (TBR0250) | Annexin Binding Buffer 10x (TBB0560) | Propidium Iodide (TBB0561).*

Before its use:

[a] Light sensitive.

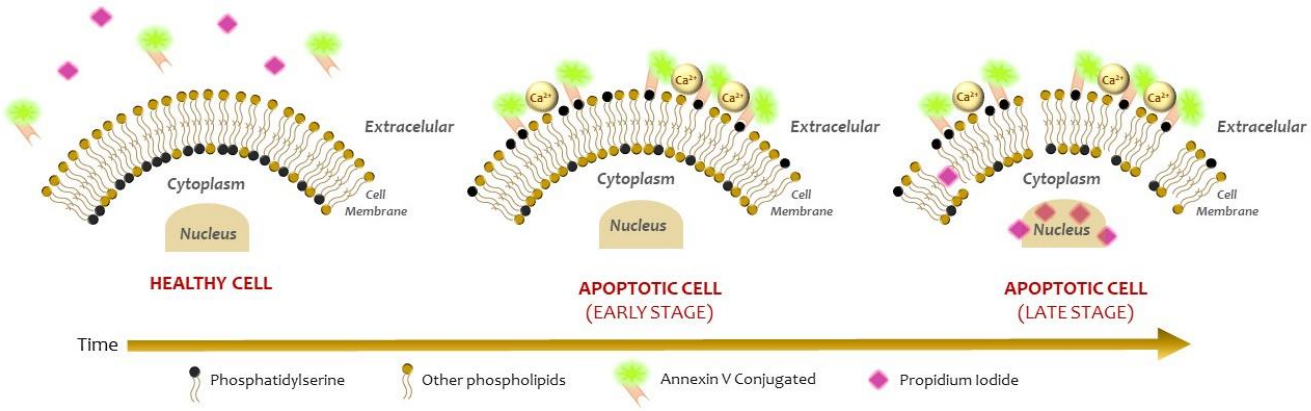
[b] Prepare fresh Annexin Binding Buffer 1x. Estimate the number of assays and prepare sufficient quantity of Annexin Binding Buffer 1x. Diluted 1 volume of Annexin Binding Buffer 10x in 9 volumes of sterile distilled water.

Storage

Store the kit at 4°C. Stable for 12 months.

Material required (not supplied)

- 5 mL Tubes.
- PBS Buffer 1x pH=7.4 (TBB0360).
- Trypsin-EDTA (*Adherent cells*).
- Serum containing media (*Adherent cells*).



Healthy cells will be negative for Annexin V and propidium Iodide staining; Early-Stage Apoptotic cells will be positive for Annexin V while Late-stage apoptotic cells or Necrotic cells will be positive for both markers.

PROTOCOL

Design your experiments including as controls: unlabeled cells, cells labeled only with Annexin V-FITC, cells labeled only with Propidium Iodide and uninduced cells.

I. LABELLING

A. FOR SUSPENSION CELLS

1. Centrifugate the culture at 500g for 5 minutes.
2. Wash cell pellet with **ice-cold PBS Buffer 1x pH=7.4**. Centrifuge for 5 minutes at 500g at 4°C, and discard the supernatant.
3. Add **Annexin V Binding Buffer 1x** to obtain final cell concentration of **1-5 x 10^6 cells/mL** and resuspend with a pipette. Keep tubes on ice.
4. Transfer **500 µL of cell suspension** in a 5 mL test tube.
5. Add **5 µL Annexin V-FITC** and **10 µL Propidium Iodide**.
6. Gently vortex the cells and incubate for 15 min at room temperature in the dark.
7. Wash cells with **Annexin Binding Buffer 1x.** Centrifugate for 5 minutes at 500g at 4°C and discard the supernatant.
8. Resuspend cell pellet in **400 µL Annexin V Binding Buffer 1x**.
9. Analyze cells by flow cytometry or fluorescence microscopy within 30 minutes.

B. FOR ADHERENT CELLS

1. Discard the culture media, leaving a small residue of media on your cells.
2. Add **ice-cold PBS Buffer 1x pH=7.4** for wash the cells. Discard the solution, leaving a small amount of PBS on the cells. Repeat this step twice.
3. Detach gently the cells with Trypsin-EDTA.

   Trypsin should be left on as short time as possible: 2-5 minutes, no longer.
4. Add serum containing media for wash the cells. Centrifugate for 5 minutes at 500g at 4°C and discard the supernatant.
5. Add **400 µL ice-cold PBS Buffer 1x pH=7.4**
6. Centrifuge for 5 minutes at 500g at 4°C, and discard the supernatant.
7. Follow the Protocol for Suspension Cells from step 3.

II. FLOW CYTOMETRY ANALYSIS

	Signal Detector	Excitation	Emission
Annexin-FITC	FL1	488 nm	518-530 nm
Propidium Iodide	FL3	535 nm	617 nm

III. FLUORESCENCE MICROCOSPY

Place the labeled cell suspension on a slide and cover with a glass coverslip. Set fluorescence microscope using FITC and rhodamine filters. Early apoptotic cells that have bound Annexin V-FITC will be showed in green while apoptotic cells will be showed an internal region red and a green halo on the cell surface.