Protocol Booklet

Product Code(s)	HB9623
Product Name	Annexin V-FITC Apoptosis Staining / Detection Kit
Purpose	Measurement of apoptotic cells in culture

Please note: This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use

Contents

Product Overview

Annexin V-FITC is a calcium dependent fluorescent probes used to identify apoptotic cells. Annexin V has a high affinity for the phosphatidylserine molecules that translocate from the inner to outer facing side of the cell membrane in cells that are undergoing apoptosis. Propidium iodide (PI) is a small molecule DNA binding fluorescent probe that is cell membrane impermeant in healthy cells. By looking at the staining pattern of Annexin V-FITC and PI it is possible to identify the cell fate:

- Cells showing Annexin V-FITC staining only are currently undergoing apoptosis.
- Cells showing both Annexin V-FITC and PI staining are either end stage apoptotic, necrotic or already dead.
- Cells showing neither Annexin V-FITC nor PI staining are currently alive and not undergoing apoptosis.

This kit is designed for use in measuring apoptosis in non-adherent cells for flow cytometry and fluorescence microscopy alongside adherent cells for fluorescence microscopy. Please note that the use of this kit for measuring apoptosis using flow cytometry in adherent cells is not recommended due to membrane damage during cell detachment.

Components & Storage

This kit contains:

- Annexin V-FITC - 0.5ml
- Propidium iodide solution - 2ml
- Annexin V binding buffer (10x) - 5ml

Note: Please ensure that all components are only used in sterile conditions to prevent contamination of the stock solutions. Store kit at 2-8°C and do not freeze.

This kit additionally requires:

- Microcentrifuge
- Sterile PBS
- 5ml sterile cell culture tubes
- Vortex mixer and flow cytometer (if analysing by flow cytometry)
- Rocker, 22mm coverslips, microscope slides, paraformaldehyde and fluorescence microscope (if analysing cells by fluorescence microscopy)

Protocol

Annexin V-FITC Assay - Flow cytometry

Annexin V-FITC is commonly used to measure apoptosis in non-adherent cells using flow cytometry. In order to set up compensation and quadrants it is recommended to set up the following control conditions:

- Unstained cells
- Cells stained with only PI
- Cells stained with only Annexin V-FITC

When inducing apoptosis it is recommended to normalise results to an untreated population due to there always being a basal level of apoptosis and necrosis in any cell population. It is also recommended to also use a positive control such as 2µM Camptothecin.

1. Culture cells using normal protocols.
2. Pellet cells through centrifugation then wash twice with ice-cold sterile PBS.
3. Pellet cells again then using a 5ml sterile cell culture tube resuspend the cells in 100µl of 1x binding buffer such that 100µl contains $1x10^5$ cells.
4. Add 5µl of Annexin V FITC and 5µl of propidium iodide to the cells, gently vortex then incubate at room temperature for 15 minutes in the dark.
5. Add 400µl of 1x binding buffer and then analyse by flow cytometry within an hour.

6. Analyse Annexin V-FITC (Excitation: 488nm, Emission: 530nm) and propidium iodide (Excitation: 535nm, Emission: 615nm) binding using suitable filter sets installed on the flow cytometer.

Annexin V-FITC Assay - Fluorescence Microscopy

Annexin V-FITC can also be used to measure apoptosis in both adherent and non-adherent cells using fluorescence microscopy. When using Annexin V-FITC in fluorescence microscopy it is always recommended to make use of both negative controls (untreated cells) and positive controls (e.g. 2µM Camptothecin)

Protocol for adherent cells:

1. Culture cells on 22mm coverslips to a density of 1x105 cells per coverslip
2. Wash cells twice with ice-cold sterile PBS.
3. Incubate cells with 500µl of 1x binding buffer
4. Add 5µl of Annexin V FITC and 5µl of propidium iodide to the coverslip then gently rock for 15 minutes at room temperature in the dark.
5. Either image cells directly or wash in binding buffer then fix with 2% formaldehyde
6. Image Annexin V FITC with a FITC filter set and propidium iodide with a RHOD filter set.

Protocol for non-adherent cells:

1. Culture cells using normal protocols.
2. Pellet cells through centrifugation then wash twice with ice-cold sterile PBS.
3. Pellet cells again then using a 5ml sterile cell culture tube resuspend the cells in 100µl of 1x binding buffer such that 100µl contains 1x105 cells
4. Add 5µl of Annexin V FITC and 5µl of propidium iodide to the cells, gently vortex then incubate at room temperature for 15 minutes in the dark.
5. Add the cells to a glass microscope slide then coverslip and image Annexin V FITC with a FITC filter set and propidium iodide with a RHOD filter set.
    i. Optional: Before adding to the microscope slide wash with 1x binding buffer then fix with 2% formaldehyde.

Guidelines, precautions, troubleshooting

Please follow the below table to resolve any problems encountered when using this kit. For any problems not listed or for any further advice please contact our technical support team at technicalhelp@hellobio.com

Problem	Potential Cause
High background	Cell density is too high. Try reducing the concentration of cells and repeat the experiment.
	Too long incubation of cells with Annexin V-FITC. Try reducing the incubation time and repeat the experiment.
	Use of overly confluent cells. Try repeating the experiment with cells at 80-95% confluency.
	Cells are contaminated (e.g. mycoplasma infection). Test cells for contamination or repeat again with a fresh aliquot of cells.
Low signal levels	Too few cells used in experiment, repeat again using a higher number of cells
	Cells did not initiate apoptosis. If a positive control was not carried out then make sure that this is included in the next experiment. Check the time course of expected apoptosis and ensure that the Annexin V-FITC is added at a time point where significant apoptosis would be expected.
	Cells were washed with PBS instead of binding buffer. Ensure that cells are only washed in binding buffer before and after addition of the Annexin V-FITC. This includes before and after fixation for adherent cells.
Erratic staining	Ensure that Annexin-V FITC staining is carried out before fixing cells. Fixation can make cell membranes leak therefore make results unreliable.
	Adherent cells used for flow cytometry experiments can be unreliable due to membrane damage during detachment. However authors such as van Engeland et al., 1996 and Casciola-Rosen et al., 1996 have reported successful use of adherent cells in flow cytometry when stained for Annexin V-FITC.

Observe safe laboratory practice and consult the safety datasheet. Please see the datasheet on our website for general guidelines, precautions, limitations on the use of the assay kit.

Contact

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