ABflo® 488 Annexin V/PI
(Apoptosis detection Kit)

Cat.No.：RK05875

Size：10T/50T/100T

Product Description

This Kit is to detect the early stage apoptotic cell. Annexin V, a 35-36 kDa Ca^{2+} dependent phospholipid-binding protein has a high affinity for phospholipid phosphatidylserine (PS), and binds to cells with exposed PS. In apoptotic cells, the membrane PS is translocated from the inner to the outer leaflet of the plasma membrane, exposing PS to the external cellular environment.

Product name ABflo® 488 Annexin V/PI & Cat.No RK05875, can detect the exposed PS through flow cytometry or fluorescence microscope.

Propidium Iodide (PI), ready-to-use nucleic acid dye, release red fluorescence light after DNA embedding. Although PI can not penetrate the entire membrane, it can penetrate necrotic cells or cells that have lost membrane integrity in the late stages of apoptosis.

Therefore, use of both Annexin V and PI, cells that are considered viable are Annexin V negative and PI negative; cells that are in early apoptosis are Annexin V positive and PI negative; and cells that are in late apoptosis or already dead are are both ABflo® 488 Annexin V and PI positive.

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ABflo® 488 has excitation of the light in 491nm and emission of the light in 516nm. PI-DNA complex has maximum absorption in 535nm and maximum emission of the light in 615nm.

Kit Component

Component	RK05875-10T	RK05875-50T	RK05875-100T
ABflo® 488 Annexin V	50μL	250μL	500μL
Propidium Iodide	100μL	500μL	1mL
Binding Buffer(10×)	5mL	25mL	50mL

Store Condition

Store at 2-8℃, valid within 1 year.

Avoid light preservation, and do not freeze.

Protocol

1. Staining

Prepare 1×Binding buffer by diluting 1 part of the 10X Binding Buffer to 9 parts of distilled water.

Centrifuge cells, centrifuge at 500-1000g for 5 min.

Adherent cells, digest cells with EDTA-free trypsin before centrifugation. To avoid false positive, do not over-digest the cells. Add cell culture medium, gently collect adherent cells,

then transfer to a centrifuge tube, and centrifuged at 500-1000g for 5 minutes to collect the cells.

Wash the cells twice with cold phosphate-buffered saline (PBS), gently vortex, collect cells after centrifugation

Resuspend cells in 1X Binding Buffer at a concentration of 1 x 10^6 cells/mL.

Transfer 100 μL of the solution (1 x 10^5 cells) to a new tube. Add 5 μL of ABflo® 488 Annexin V and 5-10 μL PI. Gently vortex the cells and incubate for 15 min at RT (25°C) in the dark.

2. Sample analysis

Flow Cytometry:

After staining and incubation, add 400μL 1×Binding Buffer to each tube, mix well, detect the cells within 1 hour by flow cytometry.

Use 3 control groups: normal cells, PI staining and ABflo® 488 Annexin V staining. Normal cells can be used as a fluorescence compensation to remove spectral overlap and set the position of the cross gate.

If the position of the cross gate is not easy to set, use apoptosis-induced cells.

Analyze and plot two-color dot plots with ABflo® 488 as the X-axis and PI as Y-axis in CellQuest.

While using both ABflo® 488 Annexin V and PI, live cells will show only a very low background fluorescence, early apoptotic cells show only strong green fluorescent, and late apoptotic cells show both green fluorescent and red fluorescent.

Fluorescence Microscope:

Add a drop of cell suspension stained by both ABflo® 488 and PI on a slide. Cover cells with a coverslip. Observe cells under a fluorescence microscope using appropriate filters for ABflo® 488 (green) and PI (red).

Troubleshooting

1. Centrifuge the reagents in the standing centrifuge tube before opening the cap.To avoid spilling liquid when opening the cap, shake the liquid in the cap to the bottom of the tube.
2. Both ABflo® 488 Annexin V and PI are light sensitive, please avoid light when storing and handling.
3. To avoid residual PBS affecting the experimental results, try to discard the supernatant in the last step of cell washing.
4. For accurate test results, suggest that samples should be analyzed within 1 hour after staining.
5. For your health and safety, always wear a lab coat, lab mask and disposable gloves.

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