## Product Information

Catalog number: REK0005

Size: 50T

Storage: Please store at -20℃ away from light, valid for 12 months.

Product components:

| NO. | Components | 50T |
|---|---|---|
| A | Propidium Iodide（20×） | 1.25ml |
| B | RNase A（50×） | 0.5ml |
| C | Staining Buffer | 25ml |

## Product Description

This product analyzes cell cycle and apoptosis based on the propidium iodide (PI) staining method. Propidium iodide is a double-stranded DNA dye that can generate fluorescence when embedded in double-stranded DNA. The intensity of fluorescence is proportional to the amount of double-stranded DNA.

In the normal cell cycle, there is one set of chromosomes in the G0 and G1 phases, two sets of chromosomes in the G2 and M phases, and the S phase is between the two. After PI staining, the fluorescence intensity of cells in different cell cycles is different. Assuming that the fluorescence intensity of cells in the G0 and G1 phases is 1, then the fluorescence intensity of cells in the G2 and M phases is 2, and the cells in the S phase are between 1 and 2.

During cell apoptosis, due to nuclear shrinkage and DNA fragmentation, DNA fragments will be lost from the perforated cell membrane during staining. When detected by flow cytometry, the fluorescence intensity is less than 1, that is, the Sub-G1 peak or the apoptotic cell peak.

Cell apoptosis can also be detected by observing the changes in cell light scattering using flow cytometry. In the early stage of apoptosis, chromatin shrinks, cell density increases, and forward angle light scattering decreases significantly. In the late stage of apoptosis, cells produce apoptotic bodies, and both forward angle light scattering and side angle light scattering decrease significantly.

## Operation steps

### 1. Cell preparation

For adherent cells:

Discard the cell culture medium, digest with trypsin, prepare single cell suspension, centrifuge at 1000 g for 5 minutes, and discard the supernatant. Resuspend the pellet with 1 ml pre-cooled PBS. Centrifuge at 1000 g for 5

minutes again, and discard the supernatant.

For suspended cells:

Collect the cell suspension, centrifuge at 1000 g for 5 minutes, and discard the supernatant. Resuspend the pellet with 1 ml pre-cooled PBS. Centrifuge at 1000 g for 5 minutes again, and discard the supernatant.

For tissue cells:

Cut the tissue block into small pieces as small as possible with scissors, and digest with 0.25% trypsin for 0.5-1 hour. Filter through a 200-400 mesh sieve to obtain a single cell suspension. Centrifuge at 1000 g for 5 minutes, discard the supernatant, and resuspend the pellet with 1 ml pre-cooled PBS. Centrifuge at 1000 g for 5 minutes again, and discard the supernatant. Note that for the last centrifugation, keep 50 μl of supernatant after discarding the supernatant and vortex to mix.

**2. Cell fixation**

Gently mix the cell pellet with 1 ml -20 ℃ pre-cooled 75% ethanol and fix it at 4 ℃ for more than 2 hours or overnight. Then, centrifuge at 1000 g for 5 minutes, gently discard the supernatant, resuspend the pellet with 1 ml pre-cooled PBS, centrifuge at 1000 g for 5 minutes, and discard the supernatant.

**3. Staining PI staining working solution preparation**

Add 25μl PI staining solution (solution A) and 10 μl RNase A (solution B) to 0.5 ml staining buffer (solution C), mix well and set aside. Add 0.5 ml of the prepared PI staining working solution to each cell sample, gently mix and resuspend the cells. Incubate at 37 ℃, away from light for 30 minutes, and directly detect on a flow cytometer (preferably within 5 hours). The excitation wavelength is 488 nm, and red fluorescence is detected. Note: The number of cells in each test should not exceed $1\times10^6$.

## Instructions

1. Centrifuge at low speed before use to prevent liquid from accumulating on the tube cap and tube wall;
2. This reagent is for research use only.
3. PI (propidium iodide) is toxic, can be absorbed through the skin, and is irritating to the eyes. Wear gloves when using it;
4. Annexin V-FITC and PI are photosensitive substances. Please avoid light when operating. When handling and labeling, try to do it in a dark place. During the incubation stage, wrap the container with aluminum foil or place it in a drawer. After cell labeling, observe with a microscope in a dark room;
5. The entire operation process should be as gentle as possible. Do not blow the cells hard. Try to operate at 4℃ to avoid affecting the cell state.
6. In the last step of cell washing, please try to discard the supernatant to avoid PBS residue, which may affect the experimental results.
7. To prevent fluorescence decay, it is advisable to perform flow detection within 1 hour.
8. If PI staining is performed for too long, the apoptosis rate may be too high. It is recommended to perform

Annexin V-FITC staining first, and then add PI staining at least 5 minutes before the machine is used.

## Notes

1. PI is toxic. Be careful when handling it, protect your eyes, avoid inhalation, and wear disposable gloves.
2. PI has quenching phenomenon. Be careful to avoid light during storage and use.

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