

Product Data Sheet

Product Name: Cell Cycle and Apoptosis Detection Kit

Cat. No.: GK10045

Components

Components	50 Tests
PI Stain Buffer	500 µL
RNase A	500 µL
Cell Stain Buffer	25 mL
Storage: -20°C, protect from light	

Protocol

This plan only provides guidance, please make modifications according to your specific needs.

1. Cell collection

(1). Suspension cells: Collect approximately 10^5 - 10^6 cells, centrifuge at 4°C and 1000g for 3-5 minutes, carefully aspirate the supernatant, add 1mL of pre cooled PBS to resuspend the cells, and transfer to a 1.5mL centrifuge tube. Centrifuge the cells again and carefully discard the supernatant. Approximately 50µL of PBS can remain to avoid removing the cells.

(2). Adhering cells: Suck out the cell culture medium and transfer it to a centrifuge tube of appropriate size. Gently wash the adherent cultured cells with PBS and add an appropriate amount of trypsin cell digestion solution to digest the cells. Incubate at room temperature until the cell shape gradually becomes round and loose, and gently shake until cell detachment occurs. Immediately add the collected cell culture medium to the digested cells to terminate digestion, gently blow down the cells, and collect them in the same centrifuge tube. Centrifuge at 4°C and 1000g for 3-5 minutes, carefully discard the supernatant, add 1mL of pre cooled PBS to resuspend the cells, and transfer to a 1.5mL centrifuge tube. Centrifuge the cells again and carefully discard the supernatant. Approximately 50µL of PBS can remain to avoid removing the cells.

(3). Tissue cells: Cut the tissue into as small pieces as possible with scissors, digest with trypsin for 0.5-1 hour, and filter through a 200-400 mesh sieve to obtain a single-cell suspension. Take about 10^5 - 10^6 cells, centrifuge at 4°C and 1000g for 3-5 minutes, carefully aspirate the supernatant, add

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1mL of pre cooled PBS to resuspend the cells, and transfer to a 1.5mL centrifuge tube. Centrifuge the cells again and carefully discard the supernatant. Approximately 50μL of PBS can remain to avoid removing the cells.

2. Cell fixation and rinsing

Add 1mL of pre cooled 70% ethanol to the cells, gently blow and mix well, and fix at 4°C for 2 hours or longer. After fixation, collect the cells by centrifugation at 1000g for 3-5 minutes, carefully aspirate the supernatant, add about 1mL of pre cooled PBS, and resuspend the cells. Centrifuge the cells again and carefully discard the supernatant.

Note:

(1). It is recommended to fix for 2 hours or more to ensure the staining effect, and fix for 12-24 hours for better results.

(2). When fixing cells, the following scheme can also be used: first add 300μL PBS to the cell pellet, resuspend and disperse the cells thoroughly, then add 700μL anhydrous ethanol and mix well to obtain a 70% ethanol fixative, which can avoid cell clustering or adhesion.

(3). For specific cells, if the cell precipitation is incomplete after fixation and centrifugation, it is recommended to extend the centrifugation time or increase the centrifugal force appropriately until the cells are completely precipitated.

(4). After cell fixation, the volume will become lighter and easier to suspend during centrifugation. PBS containing 2% BSA can be replaced with PBS to assist cell sedimentation.

(5). When removing the fixative and rinsing the cells, about 50μL of the supernatant can be retained each time to avoid discarding the cell sample.

3. Cell staining

(1). Refer to the table below and configure the staining solution according to the sample size:

	1 sample	6 sample	12 sample

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Cell Stain Buffer	0.5mL	3mL	6mL
PI Stain Buffer	10μL	60μL	120μL
RNase A	10μL	60μL	120μL
Total Volume	0.52mL	3.12mL	6.24mL

Note: It is recommended to use the staining solution immediately and prepare it on the same day.

(2). Add 0.5mL of PI staining solution to each tube of cell samples, gently resuspend the cells, and incubate at 37°C in the dark for 30 minutes. After incubation, it can be stored at 4°C or in an ice bath away from light. It is recommended to start the machine as soon as possible, preferably completing the sample flow testing within 2-5 hours.

4. Flow detection

Use flow cytometry to detect fluorescence, and perform cell DNA content analysis and light scattering analysis using appropriate analysis software. The maximum excitation/emission wavelength of PI binding to nucleic acid is 535/617nm.

Note: Before starting the machine, use a 200-400 mesh sieve to filter the cell suspension and obtain dispersed individual cells.

Background

The Cell Cycle and Apoptosis Detection Kit produced by GLPBIO can analyze the cell cycle and apoptosis status through propidium iodide staining (PI staining), and is suitable for detecting suspended cells, adherent cells, and tissue cells digested into single-cell states.

PI is a red fluorescent probe that can produce fluorescence when bound to double stranded DNA, and the fluorescence intensity is proportional to the content of double stranded DNA. PI cannot

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passively enter cells with intact plasma membranes, so the uptake and exclusion of PI can be used to distinguish between live and dead cells. PI can be excited at wavelengths between 400-600nm, with an emission wavelength of 600-700nm. The maximum excitation/emission wavelength of PI in aqueous solution is 493/636nm, and the maximum excitation/emission wavelength after binding to nucleic acid is 535/617nm.

After PI staining, assuming the fluorescence intensity of G0/G1 phase cells is 1, the fluorescence intensity of G2/M phase cells containing duplicated genomic DNA is theoretically 2, and the fluorescence intensity of S phase cells undergoing DNA replication is 1-2. When cells undergo apoptosis, due to nuclear shrinkage and DNA fragmentation, some genomic DNA fragments are lost during the staining process. After PI staining, the fluorescence intensity will be less than 1, and a sub-G1 peak will appear after flow cytometry detection, which is the apoptotic cell peak.

In addition, when cells undergo apoptosis, their light scattering also changes. In the early stage of apoptosis, chromatin shrinks, cell density increases, and the ability of cells to scatter forward angular light significantly decreases, while the ability to scatter lateral angular light increases or remains unchanged; In the late stage of apoptosis, cells produce apoptotic bodies, and the signals of forward and lateral angle light scattering decrease. Based on this kit, the DNA content and light scattering of cells can be detected by flow cytometry.

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