MCE USA
Tel: 609-228-6898
Email: tech@MedChemExpress.com

Cell Cycle and Apoptosis Analysis Kit (PI staining)

1 Components

Components	HY-K1071-50T
Cell Stain Buffer	25 mL
PI Stain (20×)	1.25 mL
RNase A (50×)	0.5 mL

2 Introduction

MCE Cell Cycle and Apoptosis Analysis Kit (PI staining) provides a convenient method to detect cell cycle and cell apoptosis. The content of DNA is changed with the process of cell cycle. DNA can be stained by fluorescent dye, such as Propidium Iodide (PI), to measure its intensity by flow cytometry to monitor the cell cycle distribution in G1/S/G2/M phase as well as apoptosis cells with signals at sub-G1 region. PI is a fluorescent intercalating agent and it binds to DNA by intercalating between the bases with little or no sequence preference. However, PI also binds to RNA, RNase A provided in this kit can eliminate RNA interference. In addition, light scattering also changes during apoptosis. In early apoptosis, light signals from Forward Scatter (FSC) become weak and signals from Side Scatter (SSC) increase or remain unchanged. When cells proceed to the phase of late apoptosis, signals from FSC/SSC both decrease.

3 General Protocol

1. Collect cells

For suspension cells: Add 10^5-10^6 cells in a 1.5 mL centrifuge tube. Centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant. Add 1 mL of pre-cooled PBS to resuspend the cells, centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant.

For adherent cells: Discard the cell culture medium, and add trypsin to dissociate cells to make a single-cell suspension. Centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant. Add 1 mL of pre-cooled PBS to resuspend the cells, centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant.

For tissue cells: Cut the tissue into as small pieces as possible, dissociate cells with trypsin for 0.5-1 h and filter through 200-400 mesh sieve. Centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant. Add 1 mL of pre-cooled PBS to resuspend the cells, centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant.

2. Fix cells

Gently resuspend the cell pellet in 1 mL of pre-cooled 70% ethanol. Store at 4°C for at least 2 hours.

Note: It is recommended to fix cells for 12-24 hours for better efficiency.

3. Wash cells

Centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant. Add 1 mL of pre-cooled PBS to resuspend the cells, centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant.

4. Prepare PI solution

Prepare appropriate amount of PI solution according to the table.

	One sample	Six samples	Twelve samples
Cell Stain Buffer	0.5 mL	3 mL	6 mL
PI Stain (20×)	25 μL	150 μL	300 μL
RNase A (50×)	10 μL	60 μL	120 μL
Total volume	0.535 mL	3.21 mL	6.42 mL

Note: The newly prepared PI solution should be stored at 4°C, protected from light, and is effective within 24 h.

5. PI staining

Add 0.5 mL of PI solution for each sample. Gently resuspend the cell pellet and incubate at 37ºC in the dark for 30 minutes.

6. Detection

Detect the fluorescence at excitation wavelength of 488 nm by flow cytometer, as well as light scattering.

4 Storage

4°C, 6 months; -20°C, 1 year

Protect from light

5 Precautions

1. Detect the fluorescence as soon as possible to avoid fluorescence quenching.
2. PI is sensitive to light, please operate away from light.
3. PI is harmful to human, take care when handling.
4. This product is for R&D use only, not for drug, household, or other uses.
5. For your safety and health, please wear a lab coat and disposable gloves to operate.