# AO/PI Double Staining Kit

## Introduction

AO/PI Double Staining Kit is a kit that can rapidly detect cell viability. This kit provides two dyes, Acridine Orange (AO) and propidium iodide (PI). AO can penetrate cell membranes, staining normal cells in green. Chromatin condensation is a hallmark of apoptosis. AO can stain condensed chromatin of apoptotic cells more brightly than normal cells. PI cannot penetrate the cell membrane and cannot stain normal cells or apoptotic cells red; for necrotic cells (whose membrane loses integrity), PI can stain them red. The staining pattern using these two dyes makes it possible to distinguish normal cells (green fluorescence), apoptotic cells (orange fluorescence) and necrotic cells (red fluorescence).

## Components and Storage

| Components | K2238-100 T | K2238-500 T |
|---|---|---|
| **AO Staining Solution** | 500 μL | 5 x 500 μL |
| **PI Staining Solution** | 500 μL | 5 x 500 μL |
| **10X Staining Buffer** | 1 mL | 5 x 1 mL |

Store the kit at -20°C, stable for 1 year. AO staining solution and PI staining solution need to be stored away from light. For frequent use, store the kit at 4°C.

## Protocol

1. **1X staining buffer preparation:** Dilute an appropriate amount of 10X staining buffer with ultrapure water to make 1X staining buffer according to the experimental needs.
2. **Sample preparation:** Harvest cells and wash cells with PBS two times. Resuspend cells with an appropriate amount of 1X staining buffer to a density of 1 x $10^6$ cells/mL.
3. **Staining:** Add 5 μL of AO staining solution and 5 μL PI staining solution to each 90 μL of cell suspension, mix gently and incubate at room temperature protected from light for 1-10 min。

***Note:** The optimal time for incubation varies depending on the cell types.

4. **Detection:** After incubation, wash cells with PBS two times. Resuspend the cells with an appropriate amount of PBS. Then detect the fluorescence signal by fluorescence microscopy or flow cytometry. AO (Ex/Em: 502/525 nm, when binding to DNA); PI (Ex/Em: 535/617 nm, when binding to DNA). If observed using

fluorescence microscopy, FITC and Cy3 filters can be used respectively.

***Note:** Detection should be performed as soon as possible after staining.

## Note

1. Detection should be performed as soon as possible after staining.
2. Fluorescent dyes are easy to quench. Please protect them from light when use and storage.
3. AO and PI are harmful to the human body, please pay attention to protection when using.
4. For your safety and health, please wear lab coats and gloves during the experiment.
5. For research use only. Not to be used in clinical diagnostic or clinical trials.

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