AO/PI Double Staining Kit

A1492198

Storage Temperature AO staining solution and AO/PI dilution buffer store at 2-8°C in dark, do not freeze; PI Staining Solution store at -20°C in dark, avoid repeated freezing and thawing.

Shipping Conditions Shipped with ice packs. Upon receipt, the product should be promptly transferred to the recommended long-term storage conditions.

Introduction

The AO/PI Double Staining Kit is a detection system based on fluorescent nucleic acid dyes, designed to quickly and reliably distinguish live, apoptotic, and necrotic cells in biological samples. Its core principle utilizes the differences in cell membrane permeability and the specific fluorescent reactions upon binding to nucleic acids of the two dyes, Acridine Orange (AO) and Propidium Iodide (PI).

AO can penetrate the cell membranes of all cell types (live, apoptotic, and necrotic). After entering the cell, AO binds to nucleic acids, but its fluorescence color depends on the target: when it binds to double-stranded DNA (dsDNA) in the nucleus, it emits green fluorescence (Ex 488 nm, Em 530 nm); whereas when it binds to single-stranded DNA (ssDNA) or RNA, it emits red fluorescence (Em >640 nm). In contrast, PI cannot penetrate cells with intact cell membranes. It only enters late apoptotic and necrotic cells, whose cell membranes have been compromised, and binds to DNA to produce red fluorescence (Ex 535 nm, Em 617 nm).

Therefore, after AO/PI double staining, observation under a fluorescence microscope allows clear differentiation of cells in different states: live cells display normal green fluorescence; early apoptotic cells show bright green speckled nuclear morphology (only AO enters); late apoptotic and necrotic cells appear orange-red or strong red fluorescence due to the combined effect of AO and PI.

Product Information

A1492198	Components	100T	500T	Storage	Quantity Per Test
A1492198A	AO staining solution	100μL	500μL	2-8℃. Store in the dark.	1μL per 0.5-1.0x10^6 cells
A1492198B	PI Staining Solution	500μL	2500μL	-20℃. Store in the dark.	5μL per 0.5-1.0x10^6 cells
A1492198C	AO/PI dilution buffer	10mL	50mL	2-8℃	0.1mL per 0.5-1.0x10^6 cells

Note: The recommended number of cells to stain per test is 0.5-1.0x10^6 cells.

Procedure

1. Preparation of Working Solution

(1) Take out the reagents and allow them to reach room temperature. Dilute the AO/PI Dilution Buffer 10-fold with double-distilled water before use.

(2) Prepare the AO/PI Staining Working Solution: The concentration of AO stock solution in this product is 10 mg/mL, and PI stock solution is 1 mg/mL. Add 50 μL of PI stock solution and 10 μL of AO stock solution to 10 mL of diluted AO/PI Dilution Buffer. Vortex to mix well, resulting in 10 mL of AO/PI Staining Working Solution.

Note: Since optimal staining conditions may vary with cell type, it is recommended that users determine the appropriate dye concentration individually based on practical conditions.

2. Staining

a. For Adherent Cells:

(a) Seed cells in culture dishes, multi-well plates, or on coverslips, and treat the cells as required by the experimental design. Gently aspirate the medium from the wells and wash with PBS for about 10 seconds, then remove the PBS.

(b) Add an appropriate volume of AO/PI Staining Working Solution, and gently swirl to ensure the dye evenly covers all cells.

Note: For adherent cells cultured in 6-well plates with a confluency over 80%, it is recommended to add 1 mL of staining working solution per well. This can be optimized according to the specific experimental setup.

(c) Incubate the cells at room temperature, protected from light, for 1-10 minutes.

(d) Aspirate the staining solution, wash 2-3 times with PBS, then add serum-free culture medium. Observe immediately under a fluorescence microscope: observe AO-DNA green fluorescence at Ex/Em = 488/530 nm, and PI-DNA red fluorescence at Ex/Em = 535/617 nm. Alternatively, analysis by flow cytometry or detection with a fluorescence microplate reader can be performed after staining.

b. For Suspension Cells:

(a) After treating the cells as required by the experimental design, count the cells. Take an appropriate number of cells, centrifuge at 500 × g for 5 minutes at room temperature, gently aspirate the medium, resuspend in an appropriate amount of PBS, and centrifuge again at 500 × g for 5 minutes at room temperature to remove the PBS.

(b) Add an appropriate amount of AO/PI Staining Working Solution to achieve a cell density of approximately 10^6 cells/mL.

(c) Incubate the cells at room temperature, protected from light, for 1-10 minutes.

(d) Place a drop directly on a glass slide, cover with a coverslip, and observe under a

microscope: observe AO-DNA green fluorescence at Ex/Em = 488/530 nm, and PI-DNA red fluorescence at Ex/Em = 535/617 nm. Alternatively, analysis by flow cytometry or detection with a fluorescence microplate reader can be performed after staining.

Note: If background interference is significant, centrifuge to remove the staining solution, wash 1-2 times with PBS, and then observe under the microscope.

Precautions

1. AO/PI staining solutions are somewhat toxic; handle with care .
2. For your safety and health, please wear a lab coat and disposable gloves.
3. Fluorescent dyes are subject to quenching; it is recommended to complete detection on the same day after staining whenever possible.