AO/PI Staining Solution

Introduction

AO/PI Staining Solution contains two types of fluorescent DNA dyes: acridine orange (AO) and propidium iodide (PI). AO is able to penetrate intact cell membranes, embed in the nuclei of all cells (including live and dead cells), and emit green fluorescence; PI, on the other hand, can only penetrate incomplete cell membranes and therefore can only be embedded in the nucleus of dead cells, emitting red fluorescence.

This staining solution is optimized for cell counting. Traditional trypan blue staining is not very specific and will also count cell debris or residual red blood cells in PMBCs, resulting in inaccurate counting results. Both AO and PI in this reagent are DNA dyes, which can effectively exclude impurities and red blood cell interference, resulting in accurate sample counting.

Components and Storage

Components	K2269-250 T	K2269-1250 T
AO/PI Staining Solution	5 mL	25 mL

For frequent use, store this reagent at 4°C away from light, and it is stable for 1 year. For long-term storage, store it at -20°C away from light.

Protocol

1. Cells collection

1) **Adherent cells:** Digest cells with trypsin, 800 rpm centrifugation for 2 min, and discard the supernatant. Resuspend the cell pellets with PBS or culture medium.

2) **Suspended cells:** 800 rpm centrifugation for 2 min and discard the supernatant. Resuspend the cell pellets with PBS or culture medium.

***Note:** If the density of cells is too high, dilute the cell suspension before starting staining.

2. Trypan Blue staining

1) Warm the AO/PI Staining Solution to room temperature.

2) Mix AO/PI Staining Solution and cell suspension in a 1:1 ratio, and incubate at room temperature in the dark for 5 min.

*Note: After mixing, count as soon as possible.

3) Add an appropriate amount of stained cells to the slide and test on a cell counter with the suitable fluorescence channel. For example, when using a 5-well slide, 20 μL is required. When using a 24-well slide, 30 μL is required.

4) Calculate the percentage of viable cells as follows:

Viable cells (%) = total number of viable cells / (total number of cells) x 100%

Note

1. This reagent needs to be protected from light, and it needs to be counted as soon as possible after staining.
2. For cell counting, it is recommended that the cell density of the sample is in the range of $1x10^4$-$3x10^7$ cells/mL to ensure the correctness of the results. If it is not within this range, it is recommended to make adjustments.
3. This reagent is carcinogenic, 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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