DeNovix Acridine Orange and Propidium Iodide Assay Protocol

Technical Note 184

Introduction

Acridine Orange (AO) and Propidium Iodide (PI) are nucleic acid dyes that combined are used to count viable and non-viable cells from tissue culture, primary cell samples or Peripheral Blood Mononuclear Cells (PBMCs).

AO is a nucleic acid-binding fluorophore that is cell membrane permeable and suitable for selective staining of nucleated living cells. PI is a nucleic acid-binding dye that is impermeable to live cells and suitable for staining dead or dying nucleated cells. All live, nucleated cells fluoresce green due to AO, and dead, nucleated cells are stained with both AO and PI and fluoresce red.

The DeNovix® AO/PI Assay and the AO/PI app on CellDrop™ Series instruments enable rapid automated cell counting for live/dead cell suspensions.

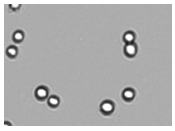
Kit Contents

Kits include a premixed solution of AO and PI in PBS. The reagents should be stored protected from light at 2 – 8°C in an airtight container.

Assay Size	Number of Tests
0.25 mL	50
1.5 mL	300

Best Practices

- Ensure that both upper and lower sample surfaces are clean, and lower the arm prior to dispensing a sample onto the measurement chamber.
- Avoid introducing air bubbles.
- Adjust the focus in the brightfield channel so that cells have bright white centers with a sharp black ring, as shown in the image to the right.



- Adjust the fluorescence exposure in the green and red channels so that cells are not over or underexposed, as described in the info ⓘ button dialog in the exposure menu.

Sample Prep

1. Equilibrate all solutions to room temperature.
2. Vortex cell suspension and AO/PI solution prior to use.
3. For each sample, mix AO/PI and a cell suspension together in a 50% solution.

   Note: A 50% solution is made up of one part AO/PI + and one part cell suspension, resulting in a Dilution Factor of 2 for the cell suspension.

4. Incubate at room temperature for 3 minutes and vortex before counting cells.

Sample Measurement

1. Ensure that the arm is in the down position, and launch the AO/PI app.
2. Clean the sample surfaces if there is visible debris in the preview image.
3. Optional: Enter a sample name and any additional sample information.
4. Select or create a protocol, and set the Dilution Factor to 2 for a 1-to-1 dye/cell suspension mix.
5. With the arm down, dispense the sample aliquot into the measurement chamber using the groove on the lower sample surface as a pipetting guide.

   Note: The volume of the sample required depends on the protocol settings for the chamber height. The required volume is displayed on the Count button.

6. Use the brightfield channel to adjust the focus.
7. Switch to the green and red channels and adjust exposure.
8. When the cells have settled and are no longer moving, tap the **Count** button.

Refer to Technical Note 186 – CellDrop Best Practices for additional guidance.

Refer to denovix.com/msds for safety data sheets for CellDrop Cell Counting Assays.

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Version: July 2019