Technical Note 184

DeNovix Acridine Orange / Propidium Iodide Assay Protocol

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 AO/PI assay stains live cells green and dead cells red to assess viability even in samples with significant debris or non-nucleated cell populations.

The DeNovix AO/PI Assay and the AO/PI app on CellDrop™ Automated Cell Counters 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^{o}C in an airtight container.

Assay Size	Number of Tests
0.25 mL	50
1.5 mL	300

Sample Volume and Chamber Height

The required sample volume for the CellDrop depends on the height of the measurement chamber, which is set in the counting protocol.

Standard Magnification (FLi & BF)

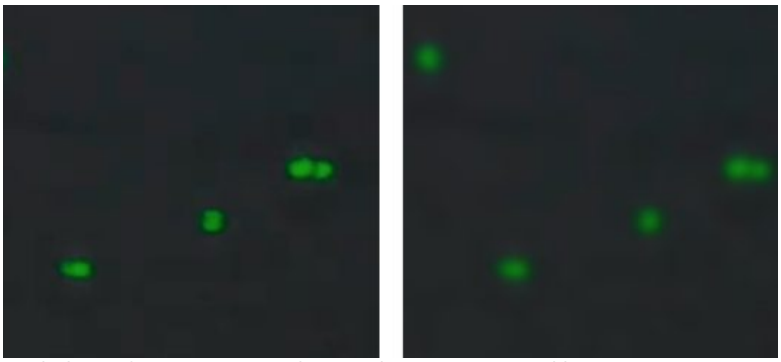
Gap Height (um)	Volume (uL)	Minimum Density (cells/mL)	Maximum Density (cells/mL)
400	40	7.0E+02	3.1E+06
100	10	2.9E+03	1.3E+07
50	5	5.9E+03	2.5E+07

Higher Magnification (FLxi & BFx)

Gap Height (um)	Volume (uL)	Minimum Density (cells/mL)	Maximum Density (cells/mL)
400	40	4.3E+03	2.6E+07
100	10	1.7E+04	1.0E+08
50	5	3.4E+04	2.1E+08

Best Practices

- Ensure that the upper and lower chamber surfaces are clean prior to loading sample.
- Lower the arm prior to dispensing sample into the measurement chamber.
- Mix the cell suspension well immediately prior to loading sample, and avoid introducing air bubbles.
- Follow the image guides to adjust focus and fluorescence exposure.
- Allow cells to settle and stop moving across the live preview before pressing the Count button.
- Adjust exposure in the green and red channels so that fluorescent cells are not over or underexposed, as shown in the info dialog in the exposure menu.



Verify Green Channel Focus. Left - good focus, Right - out of focus.

Sample Prep

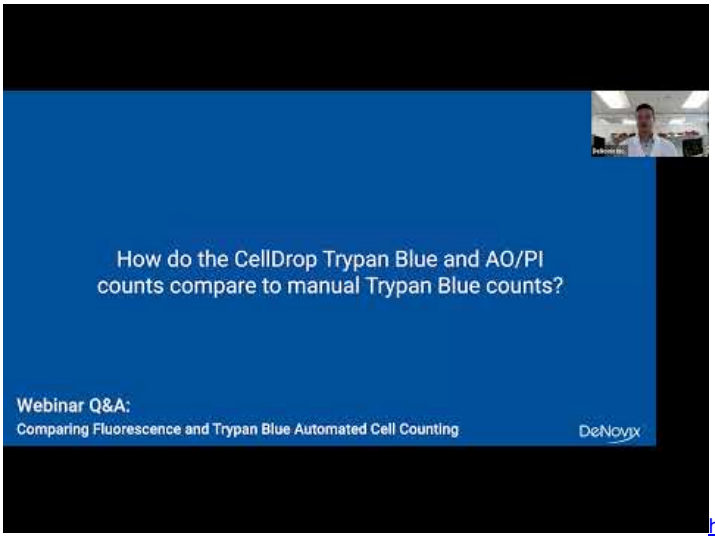
1. Mix cell suspension well. Allow AO/PI to equilibrate to room temperature and vortex briefly.
2. Mix AO/PI and cell suspension together in a 50% solution (1 part AO/PI + 1 part cell suspension = Dilution Factor of 2).
    - **Note:** There is no incubation time required. Fluorescence may start to fade if cells are in AO/PI for more than 30 minutes.
3. Mix sample thoroughly prior to loading onto the CellDrop.

Sample Measurement

1. With the CellDrop arm in the down position, launch the AO/PI app.
2. Set sample name, information and protocol as appropriate.
3. Pipette well-mixed cells + AO/PI solution and dispense appropriate sample volume into the measurement chamber, using the groove on the lower sample surface as a pipetting guide.
    - **Note:** The volume of sample required depends on the protocol settings for the chamber height. The required volume is displayed on the Count button.
4. Adjust focus according to the image guide. Set initial focus in the brightfield channel, then refine focus in the green channel.
5. Switch to green and red channels and adjust exposure according to the image guide.
6. Allow cells to settle, then press the Count button.

Refer to Technical Note 186 – CellDrop Best Practices or Technical Note 197 – AO/PI Fluorescence Assay vs. Trypan Blue for additional guidance.

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



https://youtu.be/iiIUDTJjmYQ

9-OCT-2024