Technical Note 183

DeNovix Propidium Iodide Assay Protocol

Introduction

Propidium Iodide (PI) is a red-fluorescent nuclear and chromosomal stain that only enters dead cells. Upon entry, the PI stain binds to DNA by intercalation, resulting in a red fluorescing dead cell. In aqueous solution, the dye has excitation/emission maxima of 493/636 nm. When bound, the excitation/emission maxima shifts to 535/617 nm respectively.

The PI app uses brightfield images to capture live cell counts and red fluorescence images for counting dead cells. The PI Assay and the PI app on CellDrop Automated Cell Counters enable rapid automated cell counting and viability determination for cell suspensions.

Kit Contents

Kits include a solution of PI in PBS. The PI reagent 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

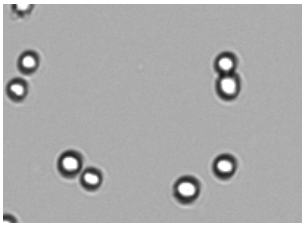
- Ensure that the upper and lower chamber surfaces are clean prior to loading sample.
- Lower the arm prior to dispensing sample into the measurement chamber.
- Mix the cell suspension well immediately prior to loading sample, and avoid introducing air bubbles.
- Follow the image guides to adjust focus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

Correct focus will show live cells with bright white centers and sharp black rings.

Sample Prep

1. Mix cell suspension well. Allow PI to equilibrate to room temperature and vortex briefly.
2. Mix PI and cell suspension together in a 50% solution (1 part 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 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 PI app.
2. Set sample name, information and protocol as appropriate.
3. Pipette well-mixed cells + 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 in the brightfield channel according to the image guide.
5. Switch to the red channel 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 for additional guidance.

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

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