



Technical Note 182

DeNovix Acridine Orange Assay Protocol

Introduction

Acridine Orange (AO) is a fluorescent dye that enters live and dead cells and interacts with DNA and RNA by intercalation or electrostatic attractions. When bound to DNA, it has an excitation maximum at 502 nm and an emission maximum at 525 nm.

Although AO enters dead cells, the intensity of the green fluorescence is very low compared to the green fluorescence emitted from live cells. This app may be useful when counting solutions containing either red blood cells and/or significant cellular debris. The DeNovix AO Assay and AO app on CellDrop Automated Cell Counters enable rapid automated cell counting for cell suspensions.

Kit Contents

Kits include a solution of AO in PBS. The AO reagent may 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

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)

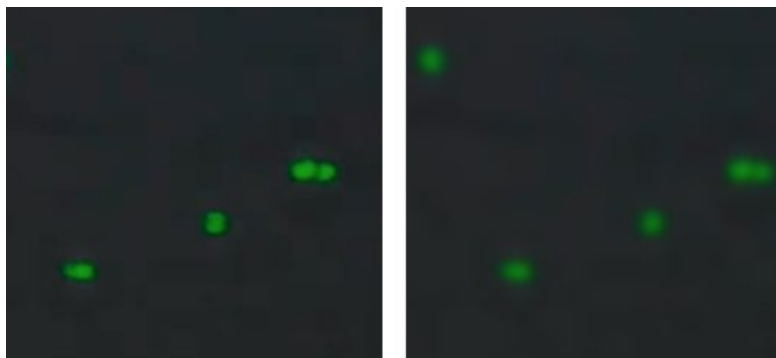
Gap Height (um)	Volume (uL)	Minimum Density (cells/mL)	Maximum Density (cells/mL)
400	40	7.0E+02	3.1E+06
100	10	2.9E+03	1.3E+07
50	5	5.9E+03	2.5E+07

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.
- Use the correct dilution factor in the protocol settings.
- 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 channel 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 to equilibrate to room temperature and vortex briefly.
2. Mix AO and cell suspension together in a 50% solution (1 part AO + 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 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 app.
2. Set sample name, information and protocol as appropriate.
3. Pipette well-mixed cells + AO 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 the green 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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