Technical Note 185

DeNovix Yeast Assay (FDA/PI) Assay Protocol

Introduction

Yeast cell live/dead staining can be done using Fluorescein Diacetate (FDA) and Propidium Iodide (PI). FDA freely moves through the plasma membrane of diverse organisms from bacteria to mammalian cells. The non-fluorescent FDA taken up by cells is converted the into the green fluorescent metabolite fluorescein. The green fluorescence is used as an indicator of live cells. PI enters only nucleated cells with compromised membranes to generate red fluorescence indicating dead cells.

The Yeast assay and Yeast app on CellDrop Automated Cell Counters enable rapid automated cell counting for live/dead yeast cell suspensions.

Kit Contents

Kits are available in a size appropriate for 300 assays and include PI, FDA and Yeast Dilution Buffer. The PI and Buffer should be stored at 2 – 8°C in an airtight container. The FDA should be stored at -20°C in an airtight container. The PI and FDA should be protected from light.

Sample Volume and Chamber Height

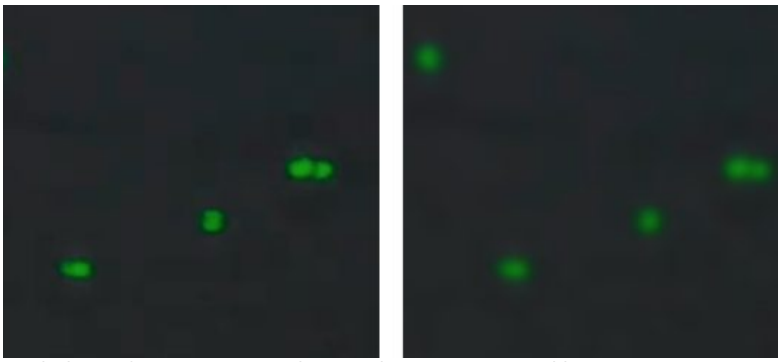
The required sample volume for the CellDrop depends on the height of the measurement chamber, which is set in the counting protocol.

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

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 and green channels so that cells are neither under or overexposed, as described in the info button dialog in the exposure menu.
- Allow cells to settle and stop moving across the live preview before pressing the Count button.



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

Sample Prep

1. Equilibrate all solutions to room temperature before use. Vortex cell suspension, FDA, and PI prior to use.
2. Spin down yeast cells and, resuspend the pellet in yeast dilution buffer.
    - **Note:** It may be necessary to further dilute cells using the yeast dilution buffer depending on density, 1:100 dilution is recommended.
3. Combine 18 µL of yeast cell sample with 1 µL of FDA and 1 µL of PI.
4. Incubate for 15 minutes at room temperature in the dark and proceed to sample measurement.

Sample Measurement

1. With the CellDrop arm in the down position, launch the Yeast app.
2. Set sample name, information and protocol as appropriate. For more concentrated yeast samples, using a chamber height of 50 µm is recommended to count higher densities and lower the settling time prior to counting.
3. Pipette well-mixed cells + FDA/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. Verify focus in the green channel prior to count.
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 for additional guidance.

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

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