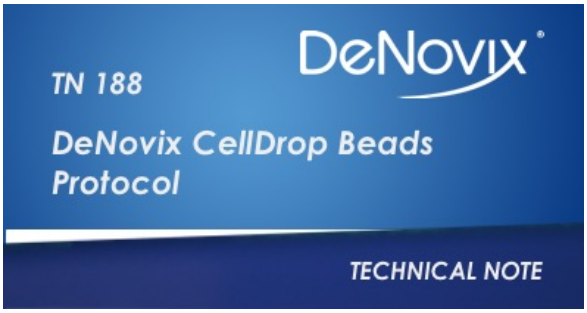

Technical Note 188

DeNovix CellDrop Beads Protocol

Introduction

DeNovix CellDrop Automated Cell Counters accurately counts cells in solutions for rapid sample quantification. Factors such as focus, exposure and concentration of particles affect the counting efficiency of the CellDrop software. Count protocol settings such as Minimum and Maximum Cell Diameter, Roundness and Fluorescence Thresholds also affect the count results according to their respective purposes.

As harvesting cells for instrument training can be costly in both time and materials, DeNovix Brightfield and Fluorescence Beads are available for evaluating the CellDrop. Instrument users can explore focus, exposure, and count protocol settings using DeNovix Bead Standards. Beads provide a convenient alternative to biologic samples for instrument qualification and training.

Kit Contents

CellDrop Brightfield Beads and CellDrop Fluorescence Beads at ~1×10^6 beads/mL are available for brightfield and fluorescence applications respectively. The beads should be stored at 2 – 8°C in an airtight container. The Fluorescent beads should be protected from light.

Brightfield Beads

The Brightfield app counts cells that have bright centers surrounded by dark rings. The Brightfield beads, when properly focused, appear as thick dark rings with a small bright center and crisp edges. These beads work well in the Brightfield app, and they will be counted as live cells in the Trypan Blue or Erythrosin B apps. The recommended protocols to use for brightfield beads are below:

Brightfield App

- Minimum Diameter: 6
- Maximum Diameter: 30
- Roundness: 55

Trypan Blue App

- Dilution Factor: 1
- Minimum Diameter: 6
- Maximum Diameter: 30
- Live Roundness: 60
- Dead Roundness: 99
- Trypan Blue Threshold: 40

Fluorescence Beads

DeNovix Fluorescent Bead fluoresce in both green and red channels. The fluorescent beads are a 50/50 mixture of red-fluorescing beads and green and red fluorescing beads with a dimmer red fluorescence. These beads may be counted in any app with fluorescence channels. The AO/PI app counts green and red fluorescing samples and is most appropriate to use with the DeNovix Fluorescent Bead Standard.

The recommended protocol for fluorescent beads is below:

AO / PI App

- Dilution Factor: 1
- Minimum Diameter: 2
- Maximum Diameter: 20
- Live Roundness: 10
- Dead Roundness: 10
- Green Fluorescence Threshold: 35
- Red Fluorescence Threshold: 35

Sample Measurement

1. Vortex bead suspensions prior to use.

2. Ensure that the arm is in the down position, and launch the appropriate app.
3. Clean the sample surfaces if there is visible debris in the preview image.
4. Set sample name, information, and protocol as appropriate. Edit the Dilution Factor in the protocol to 1 if needed.
5. Vortex beads immediately before use and dispense appropriate sample volume into the measurement chamber. Keep the arm down and use the etched groove in the lower sample surface as a pipetting guide.
    - Note: The volume of the sample depends on the protocol settings for the chamber height. The required volume is displayed on the Count button.
6. Adjust the focus and exposure according to the image guide.
7. Allow beads to settle, then press the Count button.

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

Safety data sheets are available at denovix.com/sds.

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