Technical Note 181

DeNovix Trypan Blue Assay Protocol

Introduction

The Trypan Blue exclusion assay distinguishes between live (unstained) and dead (stained) cells and enables a viability assessment of a cell suspension. Trypan Blue permeates the compromised membranes of dead cells and binds to intracellular proteins, resulting in a dark blue stained cell.

The Trypan Blue apps on CellDrop Automated Cell Counters enable rapid automated cell counting and viability of cell suspensions stained with Trypan Blue.

Kit Contents

Kits contain 0.4% Trypan Blue in PBS. The Trypan Blue reagent should be stored at room temperature (15 – 30°C) in an airtight container and does not need to be protected from light.

Assay Size	Trypan Original Concentration	Number of Tests
0.25 mL	0.4%	50
	0.2%	100
1.5 mL	0.4%	300
	0.2%	600

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

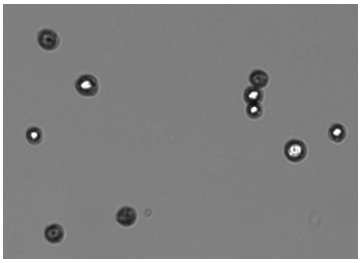
Best Practices

- 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.
- Spin down or filter Trypan Blue dye through a 0.2 µm filter to remove crystallized trypan.
- Mix cells immediately before loading sample and avoid introducing air bubbles.
- Once cells are mixed with trypan blue measure within 5 minutes.
- Follow the image guides to adjust focus and exposure so that unstained cells have bright white centers with a sharp black ring and a sharp transition from light to dark, as shown in Figure 1.
- Allow cells to settle and stop moving across the live preview before pressing the Count button.
- Optimize protocol settings for different cell types. The Default Protocol is a good starting point.

Figure 1: Correct focus and exposure settings.

Sample Prep

1. Mix cell suspension and Trypan Blue immediately prior to use.



2. Optional: Filter Trypan solution through a 0.2 µm filter to remove aggregates and crystals that can form in Trypan solution over time.
3. For each sample, mix Trypan and a cell suspension together at the desired ratio and vortex. Refer to the table below for Dilution Factor (DF) guidance examples.

Trypan Volume	Cell Volume	Protocol Dilution Factor	Recommended Exposure
5 µL 0.4%	5 µL	2	Normal
2.5 µL 0.4%	7.5 µL	1.33	Low

Sample Measurement

1. With the CellDrop arm in the down position, launch one of the Trypan Blue apps.
2. Set sample name, information and protocol as appropriate. If mixing cells and trypan in a ratio other than 1:1, edit the Dilution Factor in the protocol.
3. Pipette well-mixed cells + Trypan Blue 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 exposure and focus according to the image guide.
5. 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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