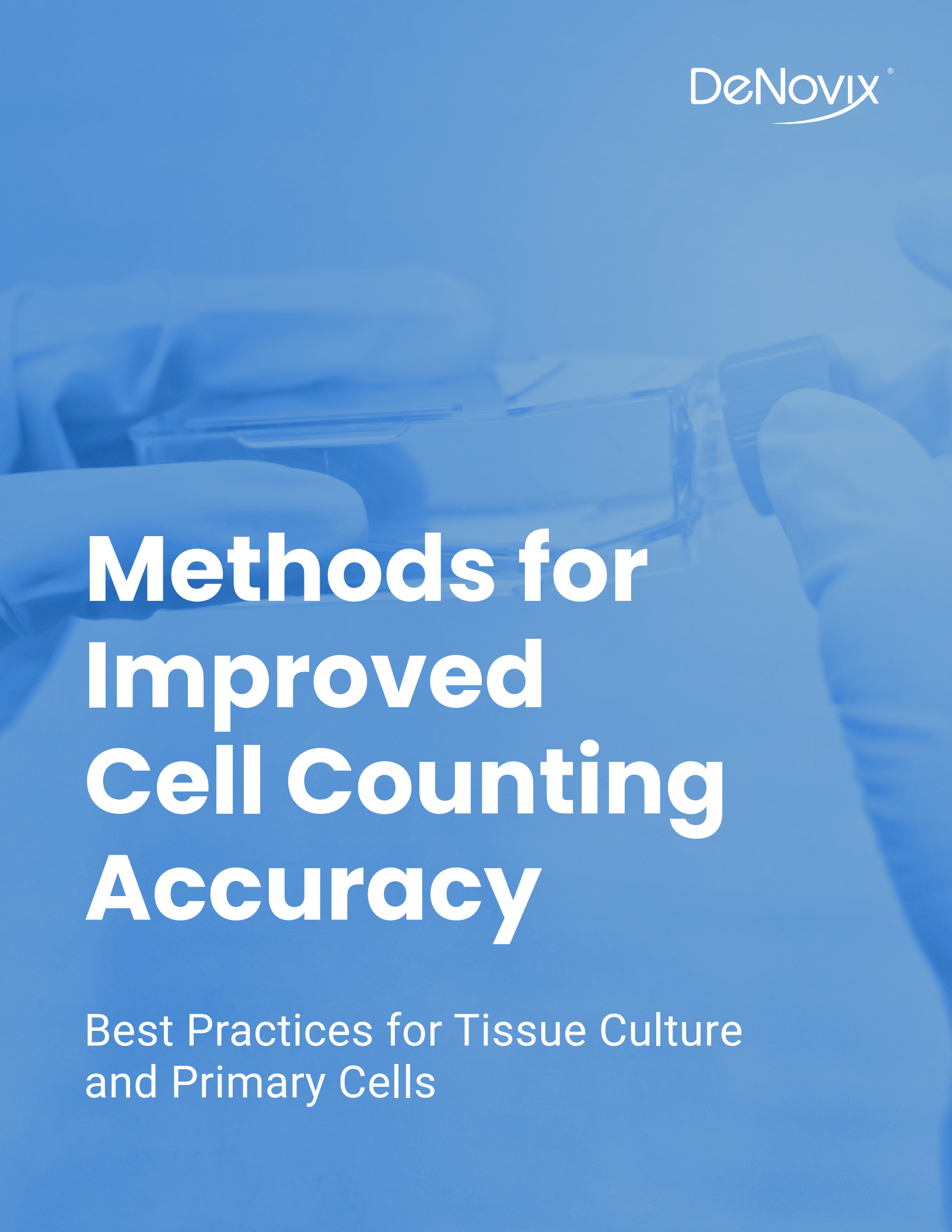
DeNovix®



Methods for Improved Cell Counting Accuracy

Best Practices for Tissue Culture and Primary Cells

What You'll Learn

Trypan Blue is widely used in cell culture labs for selectively staining dead cells and tissues. However, some sample types can present challenges for accurately assessing cell counts and viability with Trypan Blue.

For these sample types, a fluorescence assay such as **Acridine Orange / Propidium Iodide (AO/PI)** is a simple and highly accurate alternative.

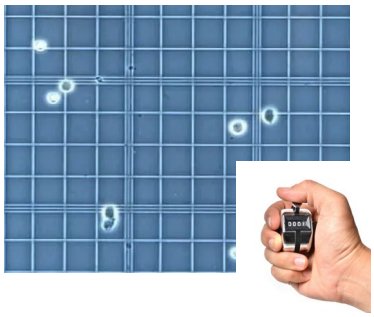
In this eBook, you'll learn about each viability method and the situations where fluorescence is advantageous.

© 2025 | DeNovix Inc.

Contents

Count and Viability Methods Discussed

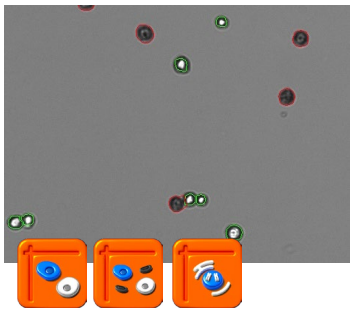
Hemocytometer + Microscope



Trypan Blue Viability Dye

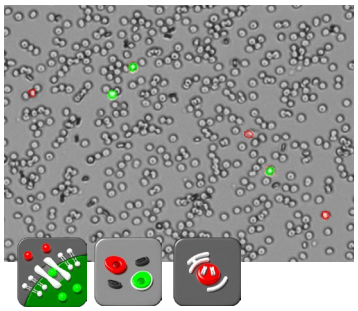
The tried and true method for cell counts and viability measurements. However, the time-consuming and subjective nature of this method can present challenges in getting accurate results.

Automated Cell Counters



Trypan Blue Viability Dye

A rapid and reliable method for tissue culture counts and viability measurements. Best when used with samples that have little or no debris present.



Acridine Orange / Propidium Iodide (AO/PI) Fluorescence Viability Dye

The combination of fluorescence automated cell counting and the AO/PI viability dye improves the efficiency, reliability and standardization of counts and viability measurements. Fluorescence methods are particularly useful in counting challenging samples, such as primary cells or nuclei.

DeNovix CellDrop™ Automated Cell Counter

The CellDrop features patented DirectPipette™ technology to eliminate plastic slides and cumbersome hemocytometers from routine cell counting. The instrument includes Dual Fluorescence and Brightfield optics, variable height sample chamber, and powerful, easy-to-use analysis software.

AO/PI Fluorescence Viability Assay vs. Trypan Blue

Trypan Blue is widely used in cell culture labs for selectively staining dead cells and tissues. The use of Trypan Blue dye can present challenges for accurately assessing cell counts and viability in some sample types. Unless additional time consuming and expensive purification steps are taken, any primary cell sample will contain debris and non nucleated cells that will be difficult to count using Trypan Blue.

The DeNovix Acridine Orange (AO) / Propidium Iodide (PI) fluorescence viability assay is an easy to use and more accurate alternative for these sample types. This technical note discusses both techniques and when fluorescence is better for viability assessments.

Trypan Blue Cell Counts

Trypan Blue works well to determine the viability of cell samples which have minimal debris. The dye is excluded from entering cells unless the membrane is damaged. The assay stains dead cells a distinctive blue color, while live cells remain transparent. The Trypan Blue apps on CellDrop Cell Counters counts live and dead cells, calculate cell concentration and report viability. The software identifies live cells as objects with a bright white center surrounded by a sharp dark ring. Stained cells are identified as an object which is dark in contrast to the background.

Trypan Blue is best suited for samples without debris, such as tissue culture cells trypsinized from a plate or flask. With debris present, identifying which objects are live cells and which are debris is challenging and subjective. Viability and counts of these samples are often overestimated when debris is counted as a cell and included in the count and viability calculation.

AO/PI Cell Counts

AO is a cell membrane-permeable nucleic acid-binding fluorophore. The dye stains the nuclei of all cells in a sample. PI is a nucleic acid-binding dye that cannot permeate live cells but is suitable for staining dead, nucleated cells.

The DeNovix AO/PI Viability Assay is a combination of the two dyes and is optimized for accurate counting and viability assessment of nucleated cells. The AO/PI apps included on CellDrop FL Cell Counters uses images from the brightfield, green fluorescence, and red fluorescence channels. All live, nucleated cells fluoresce green due to AO. Dead, nucleated cells are stained with both AO and PI and fluoresce red due to FRET quenching of the AO dye by the PI dye. Non-nucleated cells as well as cellular debris are unstained.

The AO/PI assay is excellent for analyzing nucleated cells in samples with debris present. Peripheral blood mononuclear cells (PBMCs), for example, are difficult to quantify using brightfield techniques such as Trypan Blue assay due to the high background of non-nucleated red blood cells that are not differentiated by the dye.

Conversely, measuring these samples using AO/PI is easy and accurate. Within these complex samples, nucleated cells are easily identified by green fluorescence (live) or red fluorescence (dead) against a background of non-fluorescing cells (Figure 2).

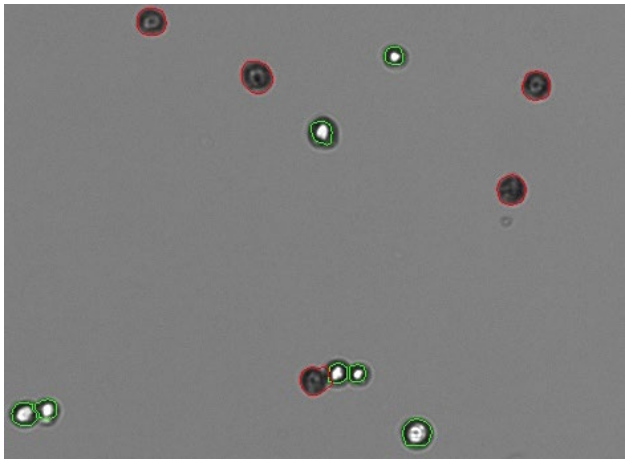


Figure 1: CHO cells counted with Trypan Blue. Tissue culture cells accurately counted with Trypan Blue.

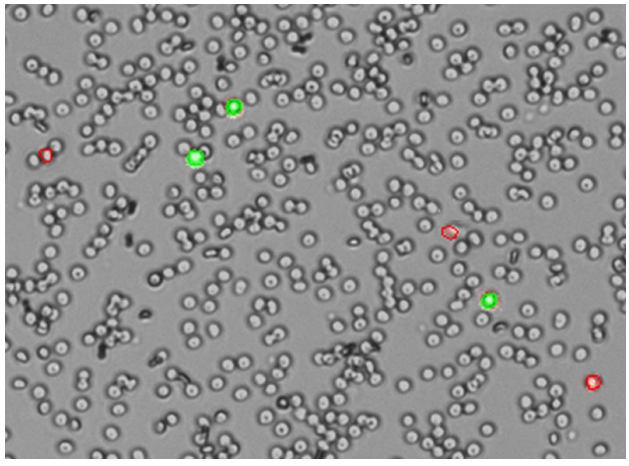


Figure 2. PBMCs counted with AO/PI. Brightfield / Green / Red image overlay for PBMCs counted with AO/PI accurately determine the viability of samples with debris.

Data Comparison for AO/PI and Trypan

Cultured Cell Sample

Chinese hamster ovary (CHO) cells were chosen as a representative model for cell samples with no debris present. CHO cells were harvested from confluent culture and centrifuged at 1000 RPM for 10 minutes. The pellet was resuspended in DMEM with 10% FBS. Initial cell density was checked with a CellDrop Cell Counter.

PBMC Sample

Mouse PBMCs were isolated from whole blood with Ficoll-Paque™ (GE Healthcare cat #17-1440-02) as a representative model of cell samples with debris present. The harvested cells were centrifuged at 1500 RPM for 15 minutes to wash the sample of remaining Ficoll solution, and resuspended in PBS. Initial cell density of nucleated cells was checked with the AO/PI assay on a CellDrop FL.

Data Summary

The initial cell density of each cell sample determined by the methods above was used as a stock to prepare 3 serial dilutions with target total cell concentrations of 1.5 × 10^6, 1.0 × 10^6 and 5.0 × 10^5 cells/mL, with dilutions labels high, medium, and low, respectively. Each dilution was counted on a CellDrop FL five times. Figures 3 and 4 shows the results of comparing counts of CHO cells and PBMCs with Trypan Blue and AO/PI.

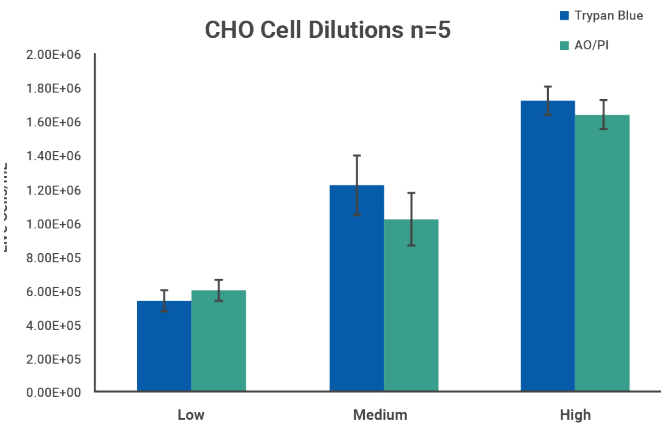


Figure 3. CHO Cells counted with Trypan Blue and AO/PI. CHO cells can be accurately counted with both Trypan Blue and AO/PI. The cell counts for each dilution are similar between counting methods.

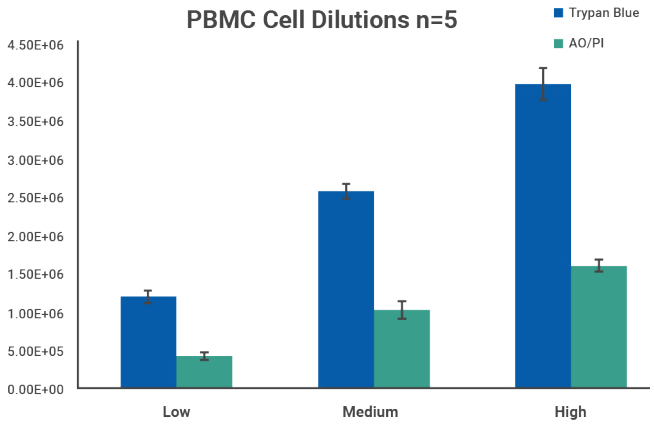


Figure 4. PBMCs counted with Trypan Blue and AO/PI. PBMCs are accurately counted with AO/PI. Cell counts are overestimated when counting with Trypan Blue due to the incorrect counting of cellular debris and non-nucleated red blood cells.

Data Comparison for AO/PI and Trypan

Automated counting and viability determination of cell samples using the CellDrop FLi removes subjectivity from the process, speeds up the workflow and enables customizable reporting and data archiving. Labs can adopt standardized methodologies that are robust against user-to-user errors and improve accuracy and reproducibility of cell counting.

Brightfield measurements using Trypan Blue are excellent for rapid reporting of cell count and viability of cell samples with minimal debris. However, the dye is subject to limitations in the ability to distinguish cells from background or debris. Dual fluorescence measurements using AO/PI enable the specific identification of live and dead cells in the presence of large numbers of non-nucleated cells and cellular debris, removing the subjectivity associated with colorimetric dyes.

AO/PI is the recommended method for these common sample types:

- Primary Cells
- Isolated nuclei
- Whole blood samples
- Samples with significant debris present
- Yeast and small cells
- Hepatocytes
- Cells with internal structure visible in brightfield

WEBINAR:

Comparing Fluorescence and Trypan Blue Automated Cell Counting

Watch Now

CellDrop Automated Cell Counter

COUNT CELLS WITHOUT SLIDES

The CellDrop™ Automated Cell Counter, engineered with DeNovix DirectPipette™ technology, is the first image-based cell counter that completely eliminates the need for slides. Simply load, count and wipe clean!

- **Sustainable** - Reduce Costs and Plastic Waste
- **Trusted** - Used by Thousands of Researchers Worldwide
- **Optimized** - Widest Range of Sample Types
- **Flexible** - DirectPipette™ (No Slides), Reusable or Plastic Slides

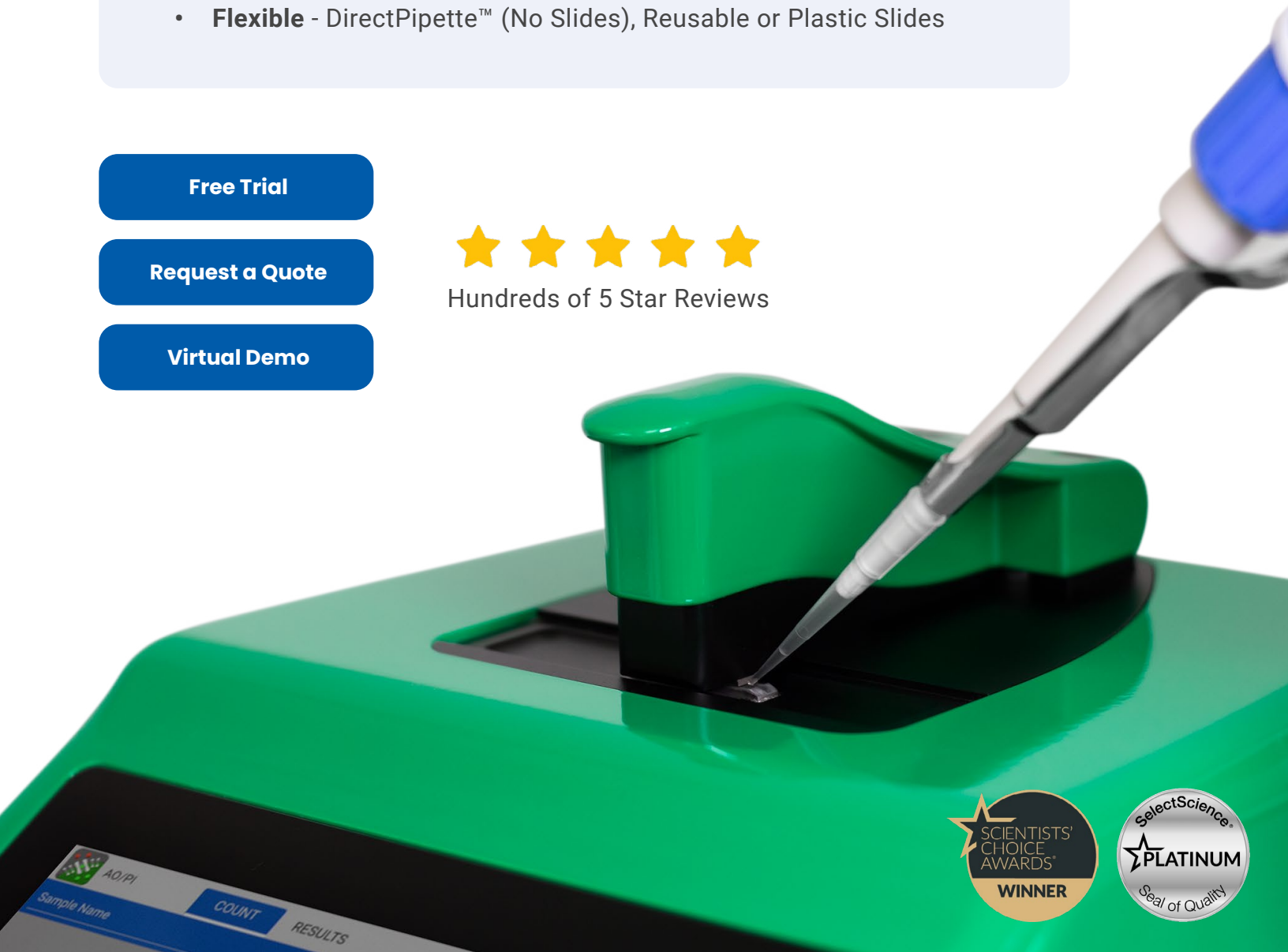
Free Trial

Request a Quote

Virtual Demo

★★★★★

Hundreds of 5 Star Reviews



SCIENTISTS' CHOICE AWARDS WINNER

SelectScience PLATINUM Seal of Quality

Counting PBMCs

It is challenging to accurately quantify and qualify the viability of primary cell samples using traditional cell counting methods. Primary cell suspensions often contain a heterogeneous collection of cell types and cellular debris as a result of digestion. Cell concentrations can also vary greatly as a result of sample location, patient or technician. Peripheral Blood Mononuclear Cells (PBMCs) are a common primary cell source used in a wide range of studies to measure immunological functions. Accurate enumeration is essential in assays such cell proliferation or cytotoxicity.

Traditional counting methods involve the use of light microscopy and hemocytometers. In addition to the inherent variability in counting between human operators, PBMCs are difficult to discern from red blood cells (RBCs). PBMCs often appear faint using light microscopy, and differentiating the biconcave shape of RBCs from leukocytes requires an experienced operator and special optical setup.

The CellDrop FLi is an automated cell counter with high performance dual fluorescence and brightfield optics. The unique DirectPipette technology eliminates disposable slides, replacing them with a wipe-clean, variable-height sample chamber.

This technical note will highlight the hardware and software features and best practices associated with counting PBMCs on the CellDrop FLi.

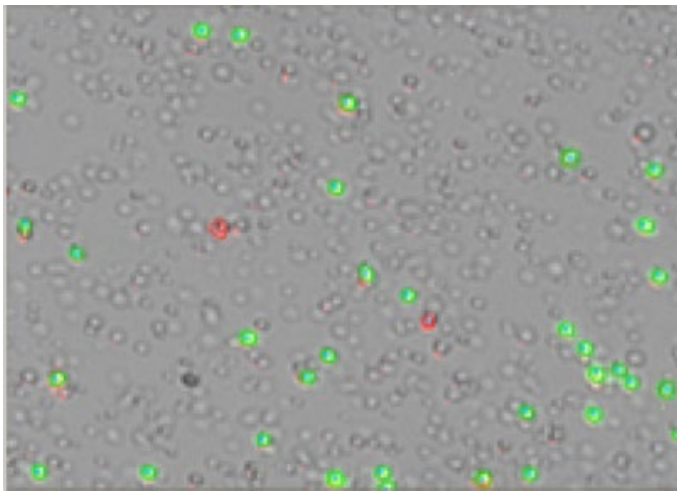


Figure 1. PBMCs Stained with AO/PI. Selective staining of live (green) and dead (red) PBMCs in the presence of RBCs (unstained).

Materials

- CellDrop FLi Automated Cell Counter
- Peripheral Blood Mononuclear Cells
- DeNovix AO/PI Assay Kit (cat #CD-AO-PI-1.5)
- Phosphate Buffered Saline (PBS)

Procedure

Cell counting and viability measurements are performed using the DeNovix Acridine Orange (AO) / Propidium Iodide (PI) Assay. AO is a nucleic acid-binding fluorophore that is cell membrane permeable and suitable for selective staining of nucleated PBMCs. PI is a nucleic acid-binding dye that is impermeable to live cells and suitable for staining dead, nucleated PBMCs.

All live, nucleated cells fluoresce green due to AO. Dead, nucleated cells are stained with both AO and PI and fluoresce red. RBCs are unstained and not counted.

1. Centrifuge PBMCs at 1000 RPM for 10 minutes, and resuspend the pellet in desired volume of PBS.
2. Vortex cells well. Dispense desired sample volume into a microfuge tube.
3. Mix sample with an equal volume of AO/PI reagent.
4. Open the AO/PI app, and select the appropriate protocol. Enter any additional sample information in the Sample Name section (optional).
5. Vortex the sample and then aspirate the sample volume indicated on the count button using a fresh pipette tip.
6. Ensure that the arm is in the down position. Rest the pipette tip in the groove on the lower sample surface and dispense the sample into the chamber. Live images will display the sample flowing evenly across the field of view. Allow the cells to settle (~10 seconds).
7. Focus on the cells using the brightfield channel. Adjust exposure for the green and red fluorescent channels as necessary.
8. Press the **Count** button located on the bottom right corner of the screen.

Cell Size and Fluorescence Thresholds

Setting an appropriate size range and fluorescence intensity for the cells of interest can exclude debris or alternative cell populations in a sample from analysis. Minimum and maximum cell diameters along with appropriate intensity thresholds can be defined and saved in protocols.

The min and max diameter can be dynamically altered using the cell size histogram once cells have been counted. The data is rapidly reanalyzed to take account of changed settings. For more advanced reanalysis, the Optimize Settings button allows the user to access and change all protocol settings on the current image and recount the sample.

Once a protocol has been optimized, the user can edit and save the original protocol with these new settings, allowing for accurate and rapid counts of future samples and standardization between different researchers.

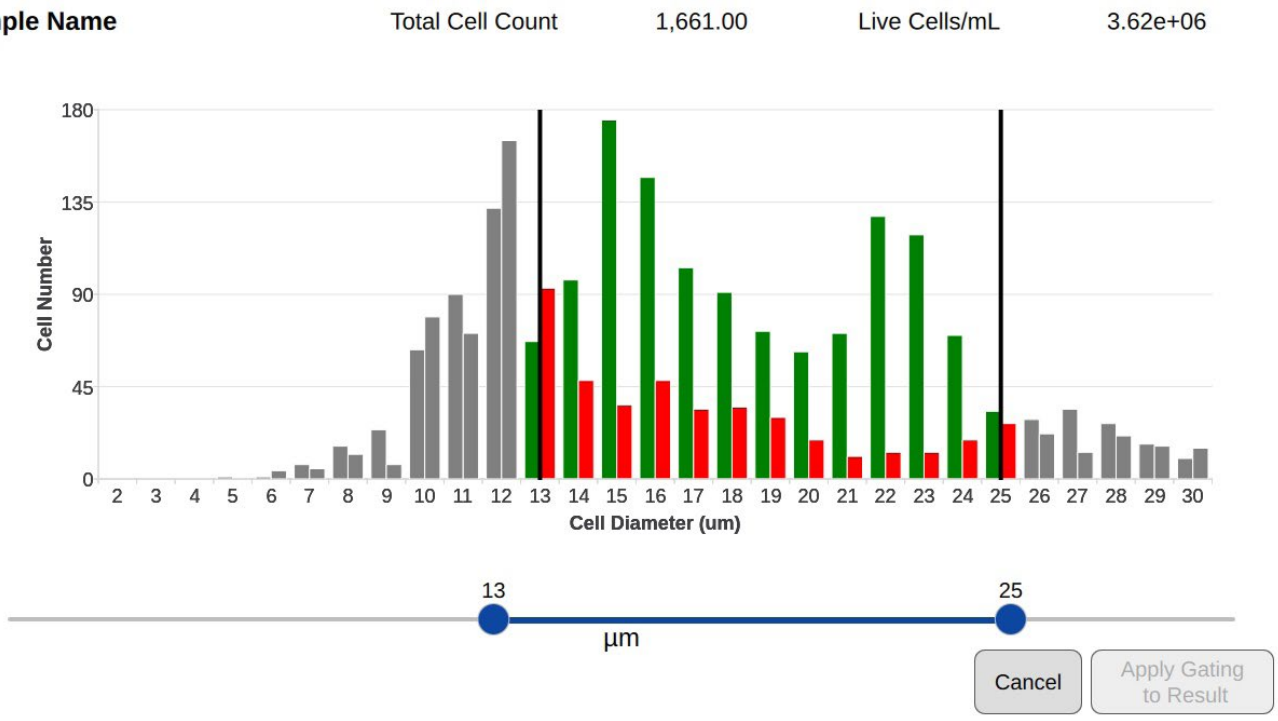


Figure 2. Cell Size Gating. Cellular debris and non-target cell groups can be excluded from analysis based on cell diameter.

Chamber Height

The CellDrop has a unique, adjustable height sample chamber that can range from 50 - 400 µm. Setting the height at 400 µm allows for a more accurate count of low density samples.

A chamber height of 50 µm allows a higher density sample to be counted without the need for further dilution. This feature gives the CellDrop the greatest dynamic range of any image based counter on the market. The chamber height can be selected from the protocols screen. The recommended concentration range at each chamber height is detailed in Table 1.

Chamber Height (µm)	Sample Volume (µL)	Min Cell Density (cells/mL)	Max Cell Density (cells/mL)
400	40	7.0×10^2	1.0×10^5
100	10	5.0×10^4	1.0×10^7
50	5	1.0×10^7	2.5×10^7

Table 1: Chamber Height Options. 100 µm is the default chamber height and is suitable for most counts.

Summary

Automated counting of PBMC samples on the CellDrop FLi removes operator variability from the process, speeds up the workflow and enables customizable reporting and data archiving. Dual fluorescence measurements using AO/PI allow the specific identification of live and dead PBMCs in the presence of large numbers of red blood cells, platelets and cellular debris.

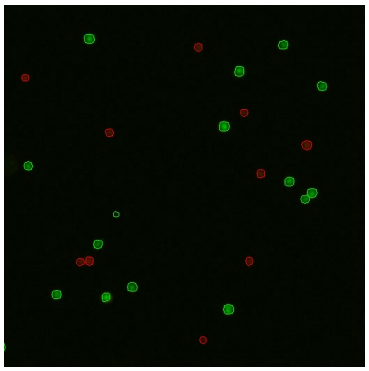
Dual fluorescence measurements using AO/PI allow the specific identification of live and dead PBMCs in the presence of large numbers of red blood cells, platelets and cellular debris.

While AO/PI are frequently used fluorophores for this application, the CellDrop is able to measure a wide range of common fluorophores. Contact our Applications Support Team at techsupport@denovix.com to discuss specific assay requirements.

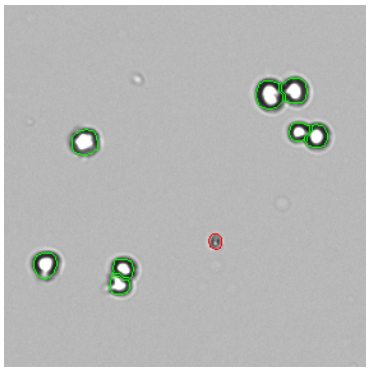
Cell Counting Assays

OPTIMIZED FOR CELLDROP™ CELL COUNTING WORKFLOWS

DeNovix CellDrop Assay Kits are simple to use and designed for Brightfield and Fluorescence applications preloaded on CellDrop instruments.



AO/PI Assay



Trypan Blue Assay

Available Assays

- AO/PI Viability Assay
- Acridine Orange
- Propidium Iodide
- Yeast
- Trypan Blue
- Erythrosin B

- Optimized for CellDrop Applications
- Cell Counting and Viability
- Rapid Incubation
- Integrated into EasyApps™ Software
- Simple Mix-and-Measure Assay

Purchase Assays Now

Request Free Sample

Viability Assays

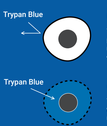
How to Select the Right Assay

What's the better method for viability assessments? Trypan Blue (TB) has long been the standard for selectively staining dead cells and tissues. But with some sample types, fluorescence assays like Acridine Orange (AO) and Propidium Iodide (PI) can provide more accurate measurements.

Trypan Blue

Trypan Blue works well to determine the viability of cell samples that have minimal debris.

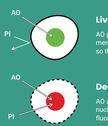
The dye is excluded from entering cells unless the membrane is damaged, so it stains only dead cells blue.



AO/PI Fluorescence

AO is a cell membrane-permeable nucleic acid-binding fluorophore that stains the nuclei of all cells in a sample.

PI is a nucleic acid-binding dye that cannot permeate live cells but is suitable for staining dead, nucleated cells.



Recommended Sample Types

Use with Trypan Blue or AO/PI | AO/PI recommended

Samples with minimal debris	Primary cells	Nuclei counting
Tissue culture samples	Whole blood samples	Yeast and small cells
	Samples with significant debris	Hepatocytes

Sample Prep

1. Optional: filter Trypan solution through a 0.2 µm filter to remove aggregates and crystals that can form in Trypan Blue
2. Add TB to cell suspension in a 1:1 ratio; mix
3. Mix sample thoroughly immediately prior to loading

1. Equilibrate all solutions to room temperature
2. Add AO/PI to cell suspension in a 1:1 ratio; mix
3. Mix sample thoroughly immediately prior to loading

Recommended Instruments & Apps

The CellDrop™ Automated Cell Counter is equipped to assess cell sample viability through both brightfield and fluorescence methods. While the CellDrop BF has applications designed for brightfield counts, the CellDrop FLi (brightfield and fluorescence) includes applications for both brightfield and fluorescence based counting.

CellDrop BF

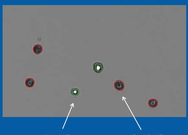
Brightfield

CellDrop FLi

Dual Fluorescence and Brightfield

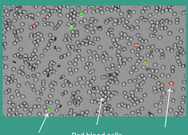
Trypan Blue CellDrop Count

Live cells viewed in brightfield exhibit a bright center with a black membrane. Trypan Blue gives dead cells a dark appearance. Live cells must be discriminated from debris either manually or by counting algorithms.

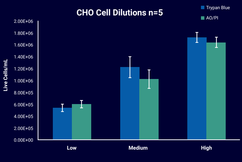


AO/PI CellDrop Count

Cells stained with AO/PI either fluoresce green (live) or red (dead). Debris and non-nucleated cells are not stained, therefore removing subjectivity from analysis.

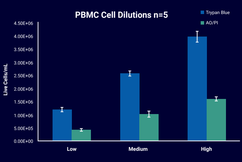


Data Comparison



CHO Cells counted with Trypan Blue and AO/PI.

CHO cells can be accurately counted with both Trypan Blue and AO/PI. The cell counts for each dilution are similar between counting methods.



PBMCs counted with Trypan Blue and AO/PI. PBMCs are accurately counted with AO/PI.

Cell counts are overestimated when counting with Trypan Blue due to the incorrect counting of cellular debris and non-nucleated red blood cells.

Summary

Brightfield measurements using Trypan Blue (or an alternative colorimetric dye, such as Erythrosin B) are excellent for rapid reporting of cell counts and viability for cultured cell lines. However, the dye is subject to limitations in its ability to distinguish cells from debris.

Dual fluorescence measurements using AO/PI enable the specific identification of live and dead cells in the presence of large numbers of non-nucleated cells and cellular debris. This removes the subjectivity associated with colorimetric dyes and improves the overall accuracy of results.

CellDrop

Using DirectPipette™ technology, the CellDrop Automated Cell Counter eliminates the need for consumable plastic slides.

This instrument features dual fluorescence and brightfield optics, a variable height sample chamber and easy-to-use analysis software.

Tech Note 197
Full comparison of Trypan Blue and AO/PI

DeNovix

© 2024 | DeNovix Inc | 16-SEP-2024

Seven Tips to Improve Cell Counting Accuracy

Cell counting and viability measurements are important for a wide range of research applications across many diverse sample types. This technical note describes seven top tips to ensure accurate and reproducible results when counting cells manually or using automated systems such as the CellDrop Automated Cell Counter.

1. Clean the Sample Surface

For every cell counting method, the sample surface must be clean. Any contamination can lead to inaccurate results. For manual cell counting, this involves removing and cleaning the glass coverslip and cleaning the counting chambers with 70% ethanol then water. When using disposable plastic slides, a new slide is required for each sample (or pair of samples if the slide has two chambers).

With CellDrop Series instruments, the chamber is formed between two optical-grade sapphire surfaces arranged parallel to one another. The sample is pipetted between the surfaces and is held in place by surface tension. To clean the surface, simply wipe it with a dry laboratory wipe (Figure 1).

For additional cleaning, flush the chamber with 15 µL of 70% ethanol or 10% bleach. After loading, let the solution sit for ~10 seconds before cleaning the surfaces with a dry laboratory wipe.

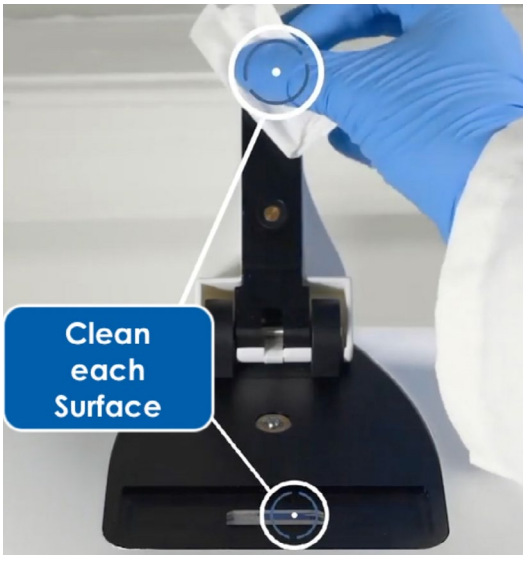


Figure 1. Clean upper and lower sample surfaces with a dry, lint-free laboratory wipe.

2. Mix Immediately Before Loading

Mixing immediately prior to loading the sample is an important step in ensuring the assayed sample is representative.

Cells of different sizes and types will settle and aggregate at differing rates. Mixing the solution immediately before loading the sample increases the probability that the aliquot will be homogeneous and accurately reflect the properties of the cell culture.

3. Minimize Cell Clumping

Cell counting requires the analysis of a small sample of the whole stock solution, so care must be taken to ensure that the sample is representative of the original stock culture. While cell counters like the CellDrop apply algorithms to identify cells within clumps, they cannot correct for a non-representative sample. When manually counting, clumps are scored more subjectively than individual cells, increasing user-to-user variability.

Extracellular DNA and cell debris following cell lysis are common causes of clumping. Cell lysis may result from factors such as overgrowth, mechanical shearing through excessive pipetting, or freeze/thaw cycles, and under- or over-digestion with trypsin can also lead to heterogeneous samples. By avoiding the causes of cell lysis and filtering samples for cell debris, cell clumps can be reduced.

4. Optimize Settings

Whether counting by hand or relying on software algorithms, it is important to adjust the focus and exposure settings to ensure the best possible visibility of cells for the most accurate results. The optimal focus and exposure will show a sharp contrast between the cell membrane and the background (Figures 3 and 4).

For fluorescence applications, the ability to individually optimize each fluorescence channel will produce the most reproducible results. Fluorescence intensities should be set to ensure that the cells are as bright as possible while remaining their true size. No light should bleed over the edges of the cells.

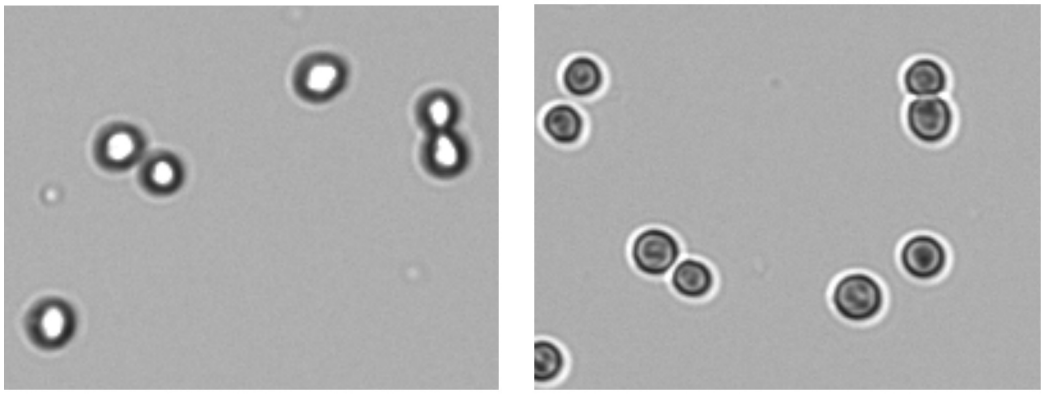


Figure 3: Optimal Focus. Correct focus (left) and poorly focused image (right).

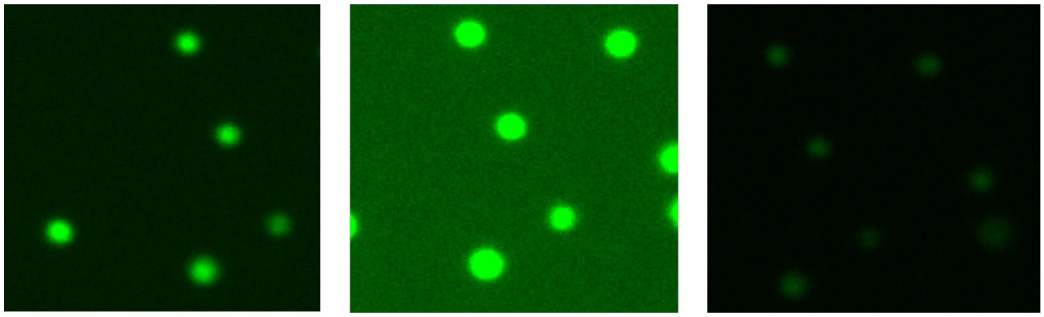


Figure 4: Exposure. Correct exposure (left), overexposed (center), underexposed (right).

5. Use an Appropriate Chamber Height

There are a range of hemocytometers available varying in depth and grid design. Take care that the correct details are used in calculations to avoid errors.

The CellDrop has a unique feature that allows for a wider range of cell density than other slide-based counters. The loading chamber height can be software adjusted to accommodate the widest range of cell density and size. On-screen guidance is given to ensure optimal height and calculations are automatically adjusted.

6. Adjust Counting Parameters

Most automated cell counters include settings that can be adjusted to best match the cells under study. For example, cell size ranges can be set and saved in order to exclude debris or other cell populations from analysis.

Settings to define cell shape are also available. In the case of the CellDrop, settings can be adjusted before or after the cells have been counted, and the data will be reanalyzed according to the new parameters.

7. Choosing Brightfield or Fluorescence

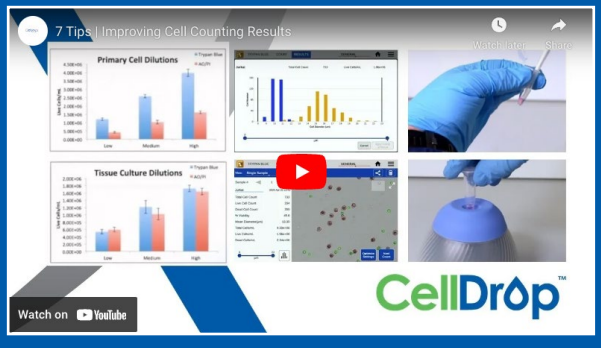
Brightfield analysis can be effective for counting cultured mammalian cells. However, it can be difficult to distinguish between live and dead cells using Trypan Blue, and for many primary cells, fluorescence is required.

For example, peripheral blood mononuclear cells (PBMCs) are usually mixed with large numbers of red blood cells (RBCs) that may show up as “dead” using brightfield analysis. However, with dyes like Acridine Orange (AO) and Propidium Iodide (PI), it is possible to stain only nucleated PBMCs and distinguish live from dead by using dual fluorescence capabilities.

AO and PI both stain nucleic acids, but only AO is capable of crossing live cell membranes. As a result, live PBMCs are stained with AO and fluoresce green, dead PBMCs are stained with both AO and PI and fluoresce red. RBCs are unstained and do not fluoresce at all.

Summary

Following these tips will help to improve cell counting accuracy and reproducibility. For more information watch the webinar below or contact the DeNovix applications team.



WEBINAR:

7 Tips for Improving Cell Counting Accuracy

Watch Now

Erythrosin B: A Less Toxic Alternative to Trypan Blue

While Trypan Blue is a well-established dye for cell counting in many labs, it is known to be carcinogenic, environmentally damaging and cytotoxic. Erythrosin B, also commonly referred to as Erythrosine, acid red 51 or FD&C red no.3, is a biosafe and non-toxic colorimetric dye that can be used as an alternative to Trypan Blue for cell counting and viability assessment.[1]

Evaluation of cell viability using Erythrosin B dye exclusion is based on a similar principle to Trypan Blue, that intact cell membranes exclude the polar molecules whereas compromised membranes do not. Live cells remain unstained while dead cells are stained and have a darker appearance.

This technical note demonstrates the use of Erythrosin B as an alternative to Trypan Blue as a cell viability stain using the CellDrop Automated Cell Counter. This technical note also compares cell counts using Erythrosin B and Trypan Blue to the DeNovix AO/PI Fluorescence assay, a combination of two dyes (Acridine Orange and Propidium Iodide) optimized for accurate counting and viability assessment of nucleated cells using green and red fluorescence channels on the CellDrop.

Results and Discussion

The accuracy of Erythrosin B as an alternative cell viability stain to Trypan Blue was determined by acquiring dead cells via starvation in PBS and then counting those cells in various mixes with live cells. Starved CHO cells with roughly 12% viability (as assessed by AO/PI) were mixed with 95% viable cells, and the results showed that determining the viability of CHO cells using Erythrosin B is as effective as using Trypan Blue or AO/PI (Figure 1) for cultured cells.

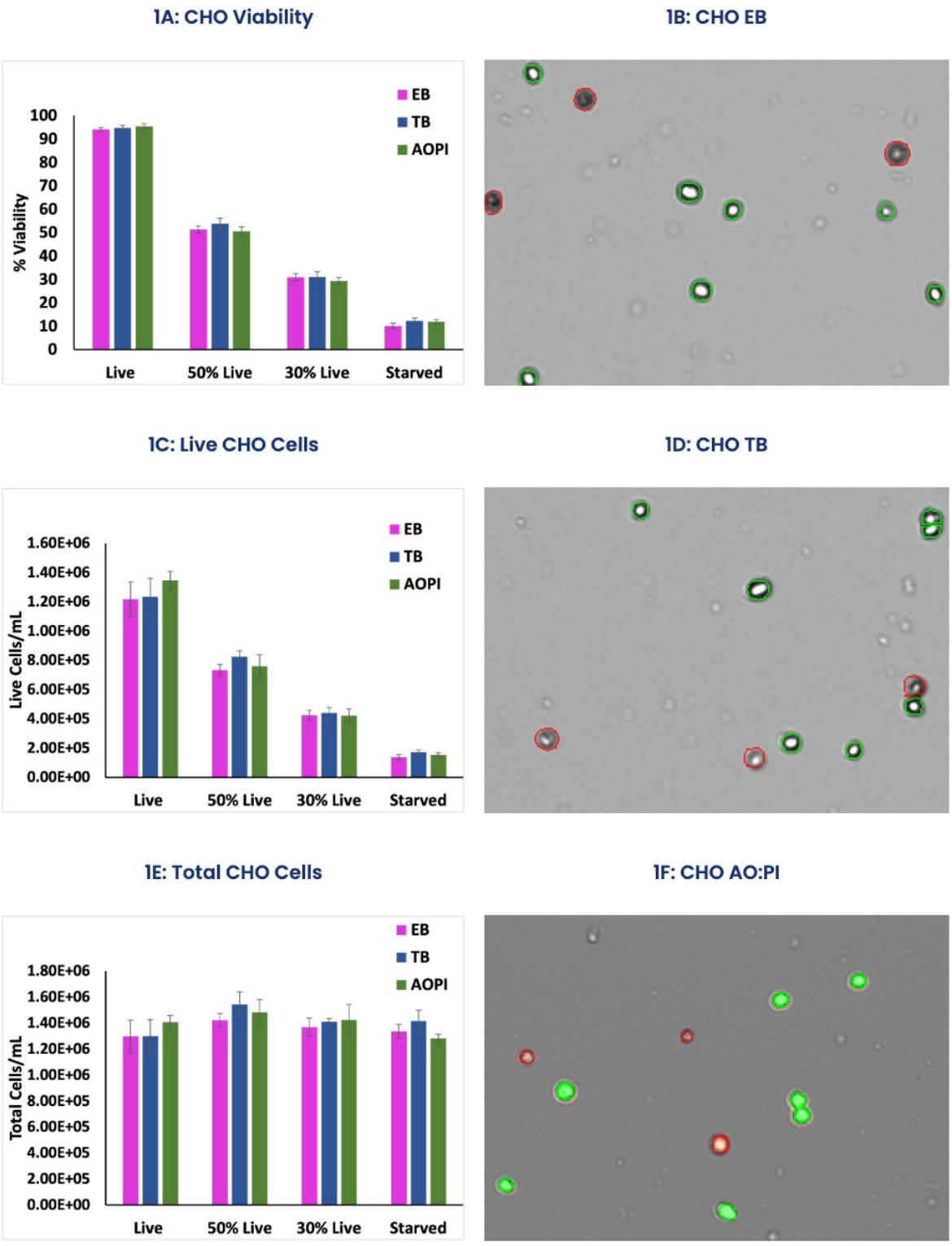


Figure 1. CHO cells counted at different viabilities as determined on the CellDrop using Erythrosin B, Trypan Blue, and AO/PI methods. (A) Cell Viability, (B) Result image of CHO cells stained with EB, (C) Live Cells/mL, (D) Result image of CHO cells stained with TB, (E) Total Cells/mL, (F) Result image of CHO cells stained with AO/PI. Each bar represents an average of six (6) replicates per group. The error bars represent standard error of the mean (SEM) of the averaged replicates. (EB = Erythrosin B, TB = Trypan Blue, AO/PI = Acridine Orange and Propidium Iodide).

Jurkat cells with less than 20% viability with a high level of debris were also mixed with highly viable cells at 70% to 30%. The results showed similar counts and viability of live, mixed and dead cells between the three (3) cell viability stains respectively (Figure 2).

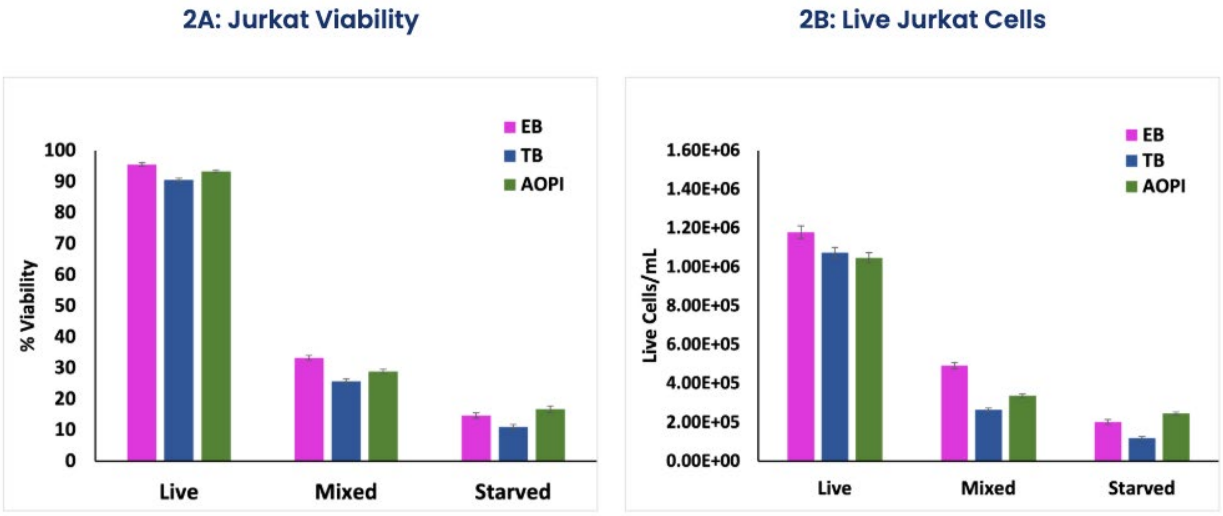
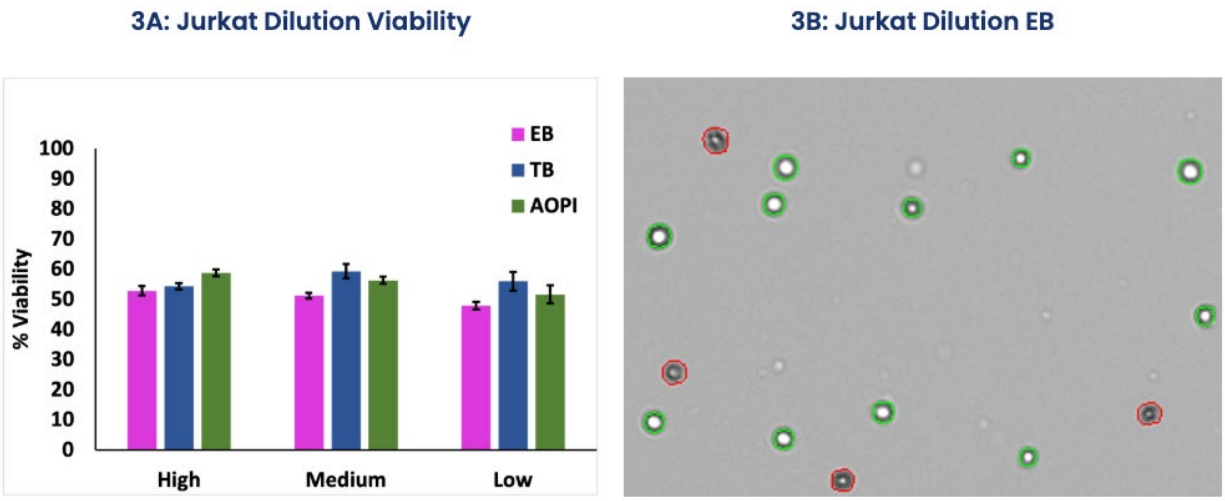


Figure 2. Jurkat cells counted at different viabilities as determined on the CellDrop using Erythrosin B, Trypan Blue, and AO/PI methods. (A) Cell Viability, (B) Live Cells/mL. Each bar represents an average of eighteen (18) replicates per group.The error bars represent standard error of the mean (SEM) of the averaged replicates. (EB = Erythrosin B, TB = Trypan Blue, AO/PI = Acridine Orange and Propidium Iodide).

Figure 3 presents viability data obtained from the dilution of a medium-low viability Jurkat passage prepared to three (3) cell densities (Figure 3). Cell viability results remained consistent across all cell densities and between the three viability stains, while the number of cells maintained the separation expected in the diluted samples.



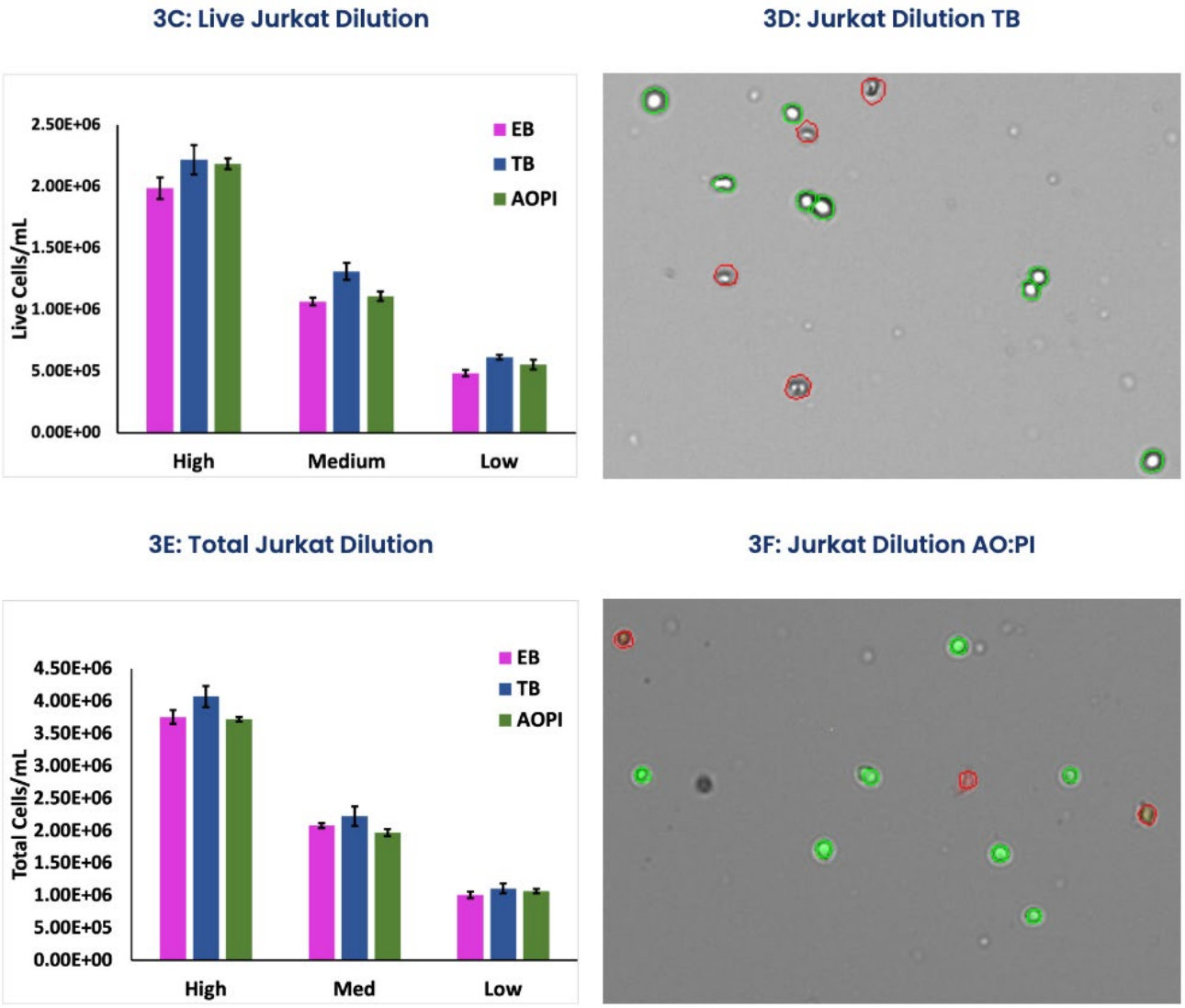


Figure 3. Medium viability Jurkat cells counted at different densities as determined on the CellDrop using Erythrosin B, Trypan Blue, and AO/PI methods. (A) Cell Viability, (B) Result image of Jurkat cells stained with EB, (C) Live Cells/mL, (D) Result image of Jurkat cells stained with TB, (E) Total Cells/mL, (F) Result image of Jurkat cells stained with AO/PI. Each bar represents an average of five (5) replicates per diluted sample. The error bars represent standard error of the mean (SEM) of the averaged replicates. (EB = Erythrosin B, TB = Trypan Blue, AO/PI = Acridine Orange and Propidium Iodide).

Materials and Methods

CHO and Jurkat cells were killed via starvation in PBS at room temperature for predetermined lengths of time specific to each cell type. The CHO cells were divided into four (4) groups: live cells, a mix of 50% live cells with 50% starved cells, another mix of 70% starved cells with 30% live cells, and the starved cell; while the Jurkat cells were separated into three (3) groups: live cells, a 70-30% ratio of starved and live cells mixed for each cell type, and the starved cell. The viability of each group was measured using Erythrosin B, Trypan Blue and AO/PI.

To confirm the accuracy of Erythrosin B at different densities compared to Trypan Blue and AO/PI, low viability Jurkat cells from a week-old passage were diluted into three concentrations (high, medium and low concentrations) and counted on the CellDrop Automated Cell Counter using Erythrosin B, Trypan Blue and AO/PI.

The three dyes under study were prepared to the following final concentrations. Trypan Blue (Sigma cat #T8154) – 0.4% W/V. Erythrosin B (Sigma cat #198269) 0.02% w/v in PBS, and AO/PI (DeNovix cat #CD-AO-PI-1.5) 0.0005% W/V. Each was used to prepare a 1:1 dilution with the cell suspension. Each dye was added to the cell sample immediately prior to counting and the sample gently mixed before loading onto the CellDrop for measurement.

The Trypan Blue app was used to count both Trypan Blue and Erythrosin B stained cells. The Normal exposure setting was used for the Trypan Blue counts. The Custom Exposure Wizard was used to automatically determine the optimal exposure for the 0.02% Erythrosin B.

The CHO Trypan Blue app protocols were used to count CHO cells stained with both Trypan Blue and Erythrosin B. Jurkat cells stained with Trypan Blue were counted using the Jurkat Trypan Blue app protocol. This protocol was modified to count Jurkat cells stained with Erythrosin B. The stained threshold was modified for Erythrosin B stained Jurkat cells from 30 to 25, the live roundness and dead roundness from 65 and 20 to 35 and 15 respectively.

The AO/PI app was used for fluorescent counts, with the standard CHO and Jurkat AO/PI protocols and recommendations applied.

Count Parameter	CHO: Erythrosin B	Jurkat: Erythrosin B
Chamber Height	100 µm	100 µm
Dilution Factor	2	2
Diameter (Minimum)	8	6
Diameter (Maximum)	30	30
Live Roundness	60	35
Dead Roundness	25	15
Stain Threshold	35	25

Summary

The data show that Erythrosin B is an effective alternative to Trypan Blue for brightfield counting of cells. Comparing these brightfield results to the fluorescent AO/PI method provides a reference against a method which is specific to nucleated cells and which also ignores debris present in the sample, showing that all three methods for viability determination are consistent for cell line samples.

The data presented indicate that Erythrosin B can reliably replace the more hazardous and environmentally damaging Trypan Blue for cell counting without the need for additional hardware.

References

1. Scott, M.F., Merrett, H.J. (1995). Evaluation of Erythrocin B as a Substitute for Trypan Blue. In: Beuvery, E.C., Griffiths, J.B., Zeijlemaker, W.P. (eds) Animal Cell Technology: Developments Towards the 21st Century. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-0437-1_178

Contact

+1.302.442.6911
info@denovix.com

DeNovix Inc.
3411 Silverside Road
Hanby Building, Suite #101
Wilmington, DE 19810

Connect

@denovix on all platforms

denovix.com/community

Explore

TN 197: AO/PI Fluorescence Assay vs. Trypan Blue

Viability Assay Infographic

TN 191: Seven Tips to Improve Cell Counting Accuracy

TN 187: Counting PBMCs

TN 226: Erythrosin B, A Less Toxic Alternative to Trypan Blue

TN 184: DeNovix Acridine Orange / Propidium Iodide Assay Protocol

TN 181: DeNovix Trypan Blue Assay Protocol

TN 230: DeNovix Erythrosin B Assay Protocol

02-JAN-2025