Viability Assays

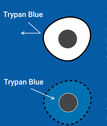
How to Select the Right Assay

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.

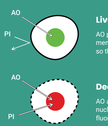
The dye is excluded from entering cells unless the membrane is damaged, so it stains only dead cells blue.



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

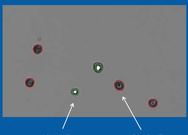
Brightfield

CellDrop FLi

Dual Fluorescence and Brightfield

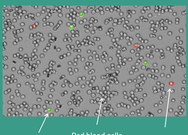
Trypan Blue CellDrop Count

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.

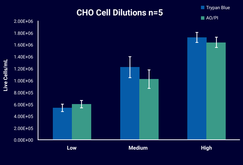


AO/PI CellDrop Count

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.

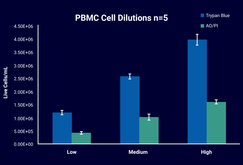


Data Comparison



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