



Technical Note 226

Erythrosin B: A Less Toxic Alternative to Trypan Blue

Introduction

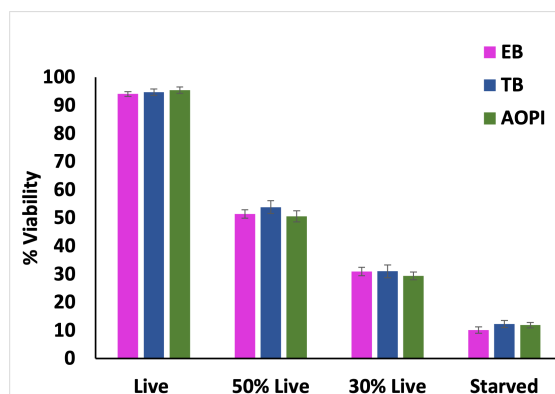
While Trypan Blue is a well-established dye for cell counting in many labs, it is known to be carcinogenic, environmentally damaging and cytotoxic. Erythrosin B, also commonly referred to as Erythrosine, acid red 51 or FD&C red no.3, is a biosafe and non-toxic colorimetric dye that can be used as an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

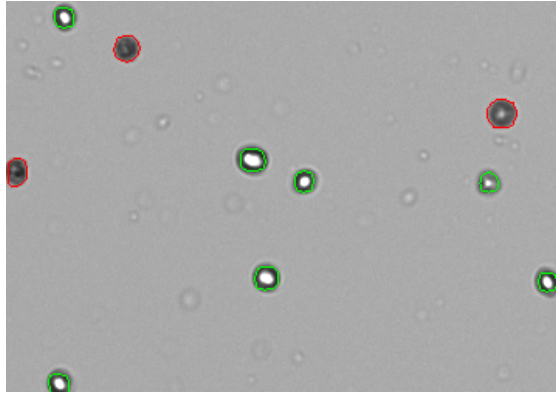
Results and Discussion

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.

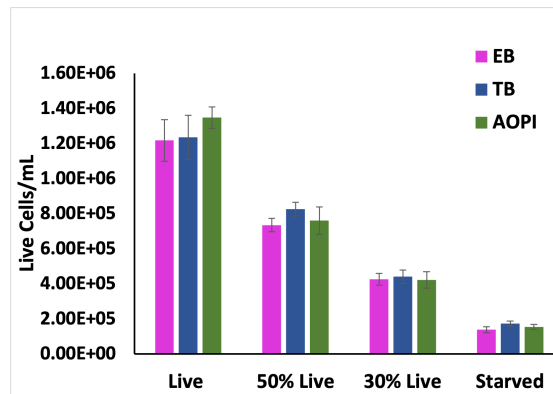
1A: CHO Viability



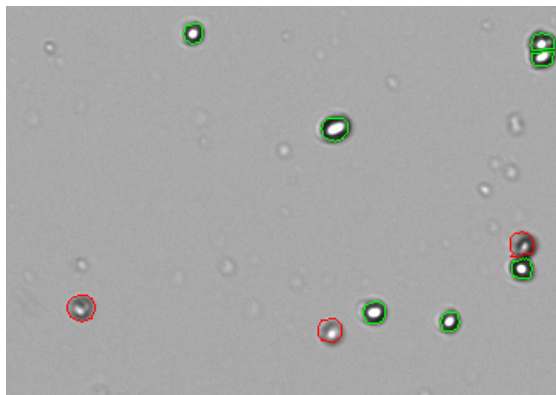
1B: CHO EB



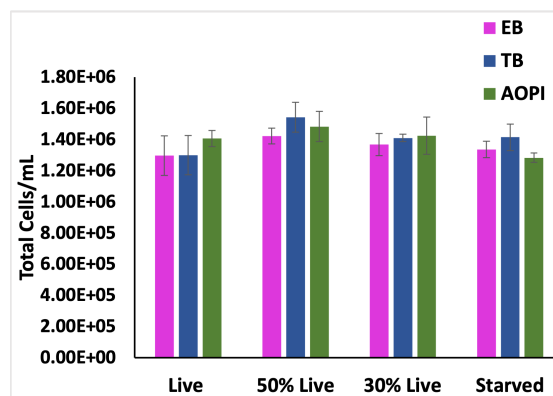
1C: Live CHO Cells



1D: CHO TB



1E: Total CHO Cells



1F: CHO AO:PI

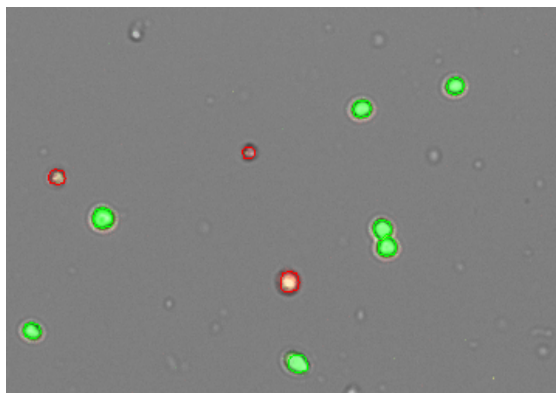
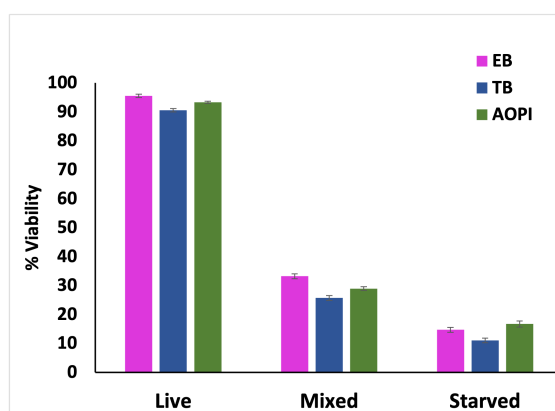


Figure 1. CHO cells counted at different viabilities as determined on the CellDrop using Erythrosin B, Trypan Blue, and AO/PI methods. (A) Cell Viability, (B) Result image of CHO cells stained with EB, (C) Live Cells/mL, (D) Result image of CHO cells stained with TB, (E) Total Cells/mL, (F) Result image 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).

2A: Jurkat Viability



2B: Live Jurkat Cells

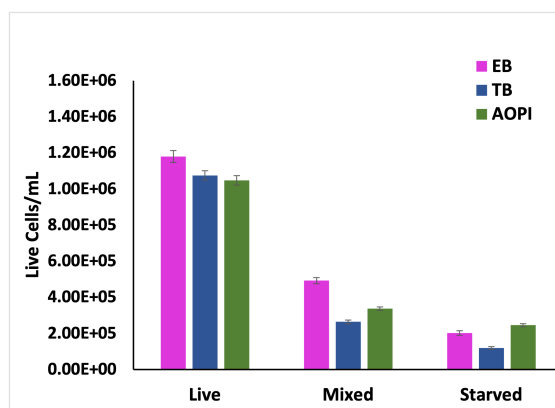
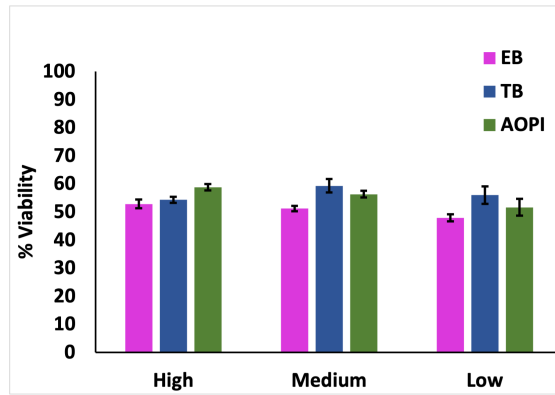


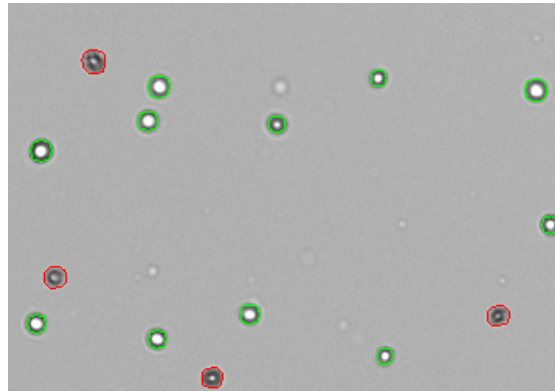
Figure 2. Jurkat cells counted at different viabilities as determined on the CellDrop using Erythrosin B, Trypan Blue, and AO/PI methods. (A) Cell Viability, (B) Live Cells/mL. Each bar represents an average of eighteen (18) replicates per group. The error bars represent standard error of the mean (SEM)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

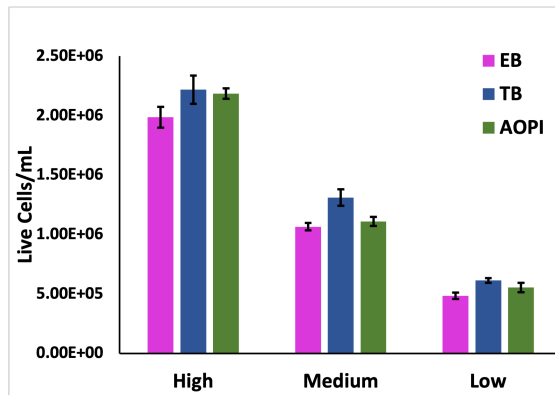
3A: Jurkat Dilution Viability



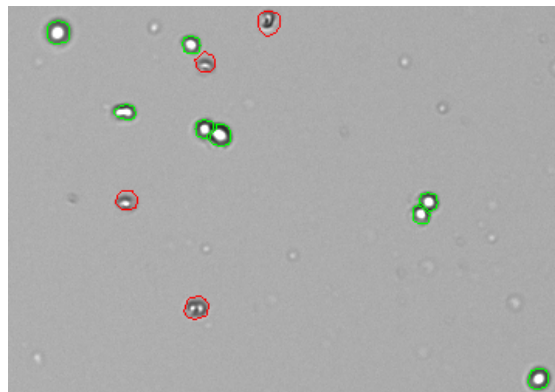
3B: Jurkat Dilution EB



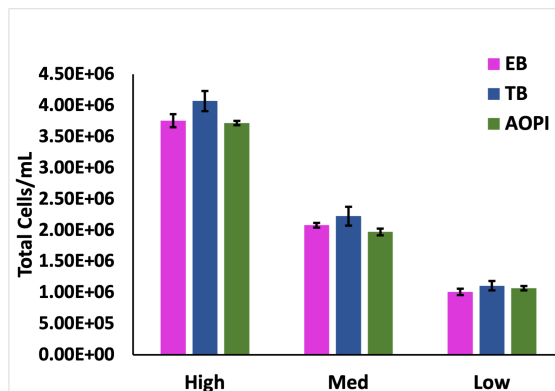
3C: Live Jurkat Dilution



3D: Jurkat Dilution TB



3E: Total Jurkat Dilution



3F: Jurkat Dilution AO:PI

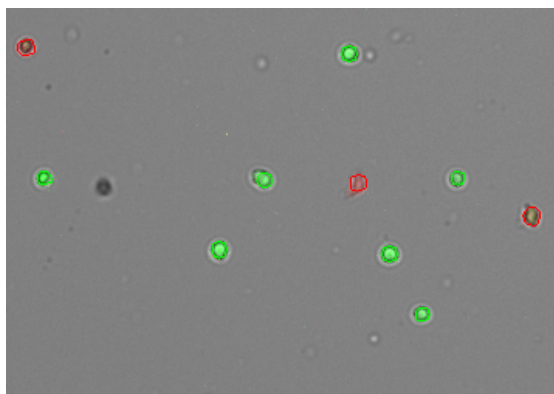


Figure 3. Medium viability Jurkat cells counted at different densities as determined on the CellDrop using Erythrosin B, Trypan Blue, and AO/PI methods. (A) Cell Viability, (B) Result image of Jurkat cells stained with EB, (C) Live Cells/mL, (D) Result image of Jurkat cells stained with TB, (E) Total 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

Count Parameter	CHO: Erythrosin B	Jurkat: Erythrosin B
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

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19-DEC-2023

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