Comparing Viability Measurements on Cell Lines Utilizing the CytoFLEX Flow Cytometer

APPLICATION NOTE

Introduction

Discriminating live cells from dead cells is important both for accurate flow cytometric analysis and for determining effects of cytotoxic drugs. Traditional methods for live/dead discrimination (7-AAD and PI) bind DNA and are not compatible with fixation and permeabilization. Zombie dyes act by binding to amine groups in the cytoplasm of dead cells while being excluded from live, membrane-intact cells[1]. Zombie dyes are available in multiple colors and are compatible with most permeabilization protocols. With four lasers and 13 colors, the CytoFlex is compatible with the use of all Zombie dyes and allows for live/dead discrimination along with surface and intracellular staining.

Methods

Cell Lines and Drugs

Cultured P12 and U937 cell lines were grown to confluence. Two million cells/mL were plated into a 24 well tissue culture plate with or without 20 mg/mL of mitomycin-c for 48 hrs at 37°Celsius in complete-RPMI medium + 5% fetal calf serum.

Viability Staining & ViCell Counting

Treated cells were washed in 10 times the volume of PBS, specifically chosen since it lacks protein, at 400g for 5 minutes. An aliquot of each cell line and treatment condition was counted on the ViCell. One million cells from each cell line and treatment condition were stained in 100 µL final volume with either 1 µL of Zombie Yellow, 1 µL of Zombie Aqua, or 20 µL of 7-AAD. All conditions were stained for 30 minutes. Then, all tubes were washed in 3 mL of growth media and spun at 400g for 5 minutes. Cell pellets were resuspended in PBS and acquired on the CytoFLEX.

Laser	405 nm					488 nm					533nm			
Filter		510	575						647					
Dye		Zombie Aqua	Zombie Yellow						7-AAD					

Results

Of the three viability dyes tested, 7-AAD produced the highest signal to noise (S:N) ratio (Figure 1). Each cell line and treatment group showed similar trends in percentage viable cells with the addition of mitomycin-c or Ly-294. Additionally, each viability dye correlated to the results generated by the ViCell (Figure 2). Although the percentages of viable cells differed slightly between the viability dyes tested, it is possible that this is due to the altering mechanisms of actions of the viability dye binding. These data suggest that it is necessary to be consistent in individual experiments with a singular viability dye of choice.

References

1- Perfetto SP, Chattopadhyay PK, Lamoreaux L, Nguyen R, Ambrozak D, Roup RA, Roederer M. Amine-reactive dyes for dead cell discrimination in fixed samples. Curr. Protoc. Cytom. Chapter 9: Unit 9.34, 2010.

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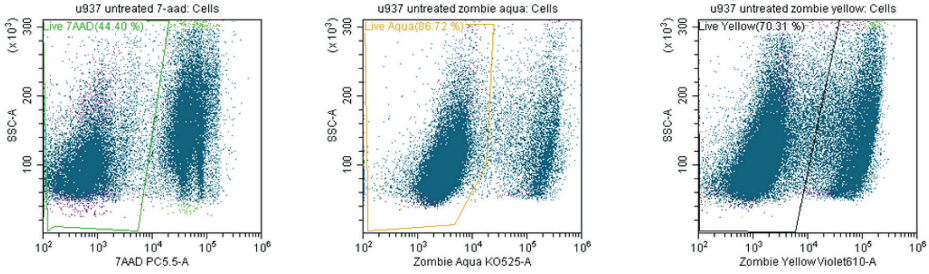
Notes

The results demonstrated in this application sheet represent those generated on the Beckman Coulter CytoFLEX Flow Cytometer. As differences exist in the performance between analyzers, the author cannot guarantee a similar appearance with the use of other flow Cytometers.

Reagent Details

Reagent	Supplier	Catalog No.
7-AAD	Beckman Coulter	A07704
Mitomycin-C	Sigma	M4287
Ly-294	Sigma	L9908
Zombie Yellow	BioLegend	423103
Zombie Aqua	BioLegend	423101

Figure 1. **Viability of U937 Myeloid Cells Measured by Multiple Dyes.**
U937 cells were stained with either 7-AAD (left), Zombie Aqua (middle), or Zombie Yellow (right) and acquired on a CytoFLEX. Ly-293 treatment results not shown.

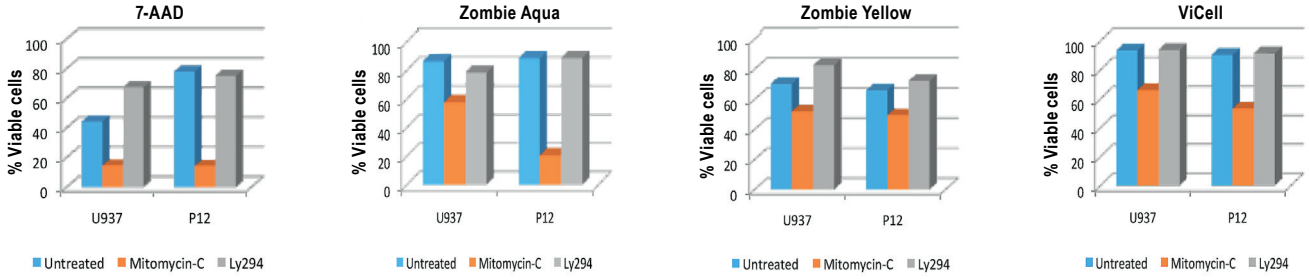


7-AAD
S:N 25.87

Zombie Aqua
S:N 16.93

Zombie Yellow
S:N 21.92

Figure 2. **Viability of U937 and P12 Cell Lines.**
U937 and P12 cell lines were incubated for 48 hours with or without mitomycin-c or Ly294.
After washing, cells were stained with 7-AAD (A), Zombie Aqua (B), Zombie Yellow (C), or counted on the ViCell. Percentage of viable cells from untreated (blue), mitomycin-c treated (orange), or Ly294 treated (gray) is graphed.



Authors

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