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Reliable, hands-free, and high-throughput cell viability assays on the MACSQuant® Flow Cytometer

Background

The determination of cell viability in any cell culture is a crucial analytical measurement for any study. This includes high-throughput cytotoxicity screens of large libraries of compounds and drug discovery research, and studies in all laboratories interested in validating their cell cultures upstream or downstream of any given application.

One of the most common ways to measure cell viability, due to the simplicity of the application, is via the determination of the cell membrane's integrity. This is done by using fluorescent dyes which are capable of penetrating the membranes of damaged/dying cells and binding to nucleic acids inside the cell nucleus. It is thought that only non-viable cells allow these dyes to enter and therefore only these cells will give a positive readout. One of the most commonly used probes for this kind of viability tests is propidium iodide (PI), which intercalates into double-stranded nucleic acids and owes its success to its low cost, rapid cell penetration kinetics, and convenient excitation and emission spectra.

To date, the majority of high-throughput cell viability assays have been performed using plate readers, due to their ease of use and the option to process entire plates very quickly. However, plate readers, while capable of providing high throughput, can only give an average of the fluorescence (and thus cell viability) of an entire well, but not at the single cell level. In contrast, flow cytometers can analyze thousands of events per well, and thus provide detailed high-content information on the viability and phenotype of single cells as well as the cell count of the entire well at the same time.

However, regardless of their processing power, conventional flow cytometers have been limited in their use for high-throughput cytotoxicity assays due to the inherent nature of dyes such as PI to penetrate the membranes of healthy, and not only dead cells, during longer exposure times[1]. This skews the results of cytotoxicity experiments during extended plate processing times. To date, most of the protocols for assessing cell viability with PI still advise users to add PI manually only seconds or minutes before acquisition in order to control for any and all cytotoxic/penetration effects of PI.

	Manual well-to-well staining	Pre-staining all samples before acquisition	MACSQuant Autolabeling
Hands free		●	●
Reproducible			●
High throughput		●	●
Short PI incubation times	●		●

Table 1: Features of different PI staining procedures. Short PI incubation times minimize non-specific staining.

MACSQuant® Flow Cytometers (MACSQuant Analyzer 10, MACSQuant VYB, and MACSQuant X) feature an integrated robotic pipetting arm which can automatically pipet fresh PI directly into each sample and standardize the incubation and labeling process. This, together with the instrument's automated sampling of 96-well plates, provides a robust, automated, hands-free, and high-throughput setup for all cell viability experiments (table 1).

Methods

To validate the importance of using an automated PI staining process with a MACSQuant Flow Cytometer, we processed various 96-well plates of Jurkat cells under different conditions. In detail, Jurkat cells (1×10^6 cells/mL) were either incubated with concentrations of 100 nM or 200 nM of staurosporine (a cytotoxic drug) or left untreated (control) for 18 h at 37 °C. After the incubation, all wells were washed with fresh medium. Plates were then treated in two different ways: i) all wells were pre-stained simultaneously with a 1:10 dilution of a PI solution (100 µg/mL) using a multichannel pipette before acquisition on a MACSQuant Analyzer 10, or ii) wells were treated via autolabeling, where a 1:10 dilution of PI was added to each well individually, with a 2-minute incubation time per well, using the autolabeling function of the MACSQuant Analyzer 10 during cell processing. All plates were analyzed on the MACSQuant Analyzer 10 using 20 µL uptake volume at high flow rate. All samples were individually resuspended by using the MACSQuant Analyzer's integrated aspiration mixing.

Results

Figure 1 shows that samples from wells in which PI was pre-stained gave statistically higher MFI values on the B3 channel (where the PI signal was detected) when compared to wells where PI was added using the autolabeling feature of the MACSQuant® Analyzer 10. Furthermore, all the samples from the pre-stained wells showed a much higher degree of variability compared to the samples treated by autolabeling. This effect is most likely due to the passive entrance of PI across the membranes of not only non-viable but also viable cells during the extended plate processing time. Another important finding was that while a statistical difference was seen between the 100 nM autolabeling vs. 200 nM autolabeling groups, this was not the case between the 100 nM vs. 200 nM pre-stained groups. This was most probably due to the increased saturation of the B3 channel as a result of high exposure times to PI, thus not allowing a proper discrimination of the cytotoxic effect of staurosporine at different concentrations.

These results indicate that pre-staining a plate with PI, while remaining a relatively "hands-free" process (it still requires the user to manually handle PI), does not guarantee adequately standardized or trustworthy results. The non-specific staining makes it difficult to distinguish real-positive dead cells from false-positive "healthy" cells which have had PI leak across the cell membrane. In contrast, the autolabeling function of the MACSQuant Analyzer provides standardized, specific PI labeling for reliable cell viability assessments.

Conclusion

The integrated robotic pipetting arm of the MACSQuant Flow Cytometers enables:

- automated staining of up to 96 samples at a time
- a standardized high-throughput method of assessing cell viability using PI
- consistent results in a completely hands-free setup

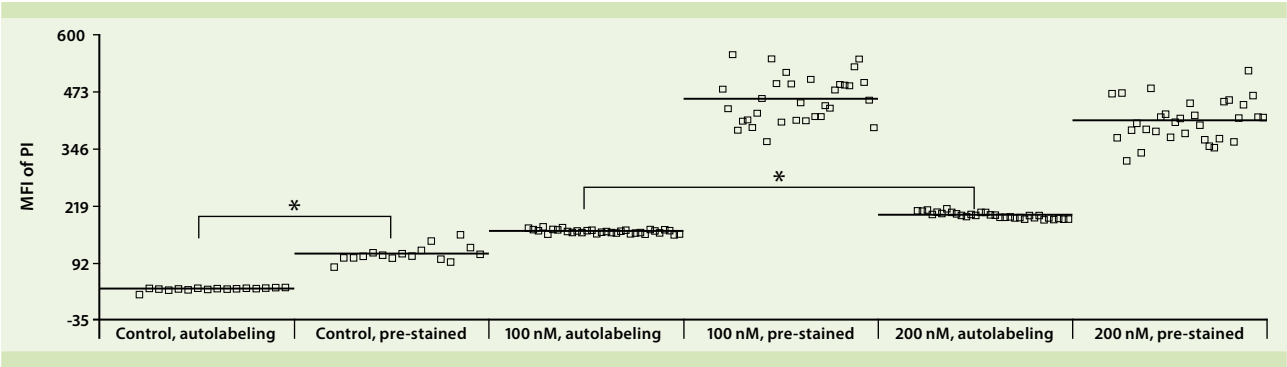

Figure 1: Jurkat cells were incubated in 96-well plates in the absence (control) or presence of 100 nM or 200 nM staurosporine for 18 h at 37 °C and washed with fresh medium subsequently. All wells of a plate were then either pre-stained simultaneously with PI, using a multichannel pipette, or treated via autolabeling as indicated in the methods section. Data from all plates were generated on a MACSQuant Analyzer 10 and the mean fluorescence intensity (MFI) of PI was analyzed. A two-tailed unpaired t-test analysis was performed between the different groups. The asterisk indicates a statistically significant difference at $p<0.05$. All flow cytometry analysis and statistical reporting was done using Flowlogic™ Software.

Reference

1. Zhao, H. *et al.* (2010) Rationale for the real-time and dynamic cell death assays using propidium iodide. Cytometry A 77: 399–405.

MACS® Product	Order no.
MACSQuant Analyzer 10	130-096-343
MACSQuant VYB	130-096-116
MACSQuant X	130-105-100*
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Flowlogic V7 Annual License Key – Academic	160-002-087

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