MACSQuant® X Analyzer facilitates Ca^{2+} flux assays

Background

Intracellular calcium (Ca^{2+}) serves as a crucial second messenger regulating a variety of physiological processes such as cell division, muscle function, neurotransmission, and vascular contraction[1]. Upon specific stimulation, intracellular Ca^{2+} concentrations can quickly and significantly increase by the release of internal Ca^{2+} storages or by entry from the extracellular space through ion channels[2]. Measuring this Ca^{2+} flux using fluorescent Ca^{2+}-binding indicators is a widely used method to study cellular responses and to screen receptor or ion channel agonist and antagonist compounds[3]. Flow cytometry–based Ca^{2+} flux assays can rapidly analyze large numbers of cells at the single-cell level and allow high-content analysis through additional staining to e.g. discriminate dead cells and debris that might interfere with the assay.

Here we show that the MACSQuant® X Analyzer facilitates Ca^{2+} flux assays and allows continuous data acquisition throughout the assay. Its peristaltic pump aspirates cells continuously and the open system enables adding of stimuli without pausing the experiment and the measurement.

The MACSQuant X Analyzer has no limited sample volume and is compatible with single tubes or the MACSQuant X 5 Rack carrying up to 24 samples so that multiple stimuli and inhibitors can be tested within one assay.

Materials and methods

Jurkat cells were incubated with 8 µM eFluor514™ Calcium Sensor Dye at 37 °C for 30 min and stored at 4 °C until measurement (no wash required). After calibration of the MACSQuant X Analyzer, the instrument was set to the standard mode with medium flowrate (compensation is not required if no additional staining is performed). For the control, uptake volume was set to 50 µL and acquisition was started. For Ca^{2+} stimulation, uptake volume was set to 200 µL. Cells were acquired for at least 15 seconds to measure baseline levels, before 25 µL of ionomycin (5 µg/mL) were added. Acquired data were analyzed and plotted with MACSQuantify™ Software.

Results

First, Jurkat cells were pre-gated to exclude debris resulting in a pool of single Jurkat cells that were selected for further downstream analysis (fig. 1).

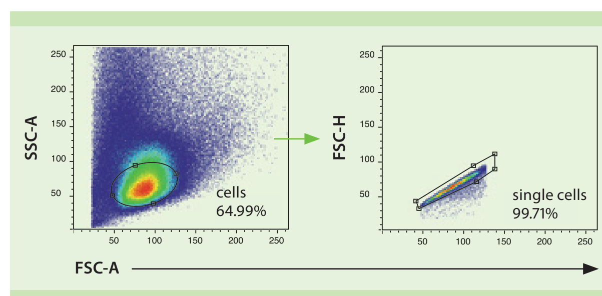


Figure 1: Gating strategy for the identification of single Jurkat cells free of debris.

Baseline eFluor 514™ fluorescence intensities of unstimulated and stimulated Jurkat cells had similar levels (fig. 2). Addition of ionomycin quickly increased the intracellular Ca^{2+} concentration as indicated by a strong increase in eFluor 514™ fluorescence intensity that gradually dropped down with time (fig. 2 A and B, right plots).

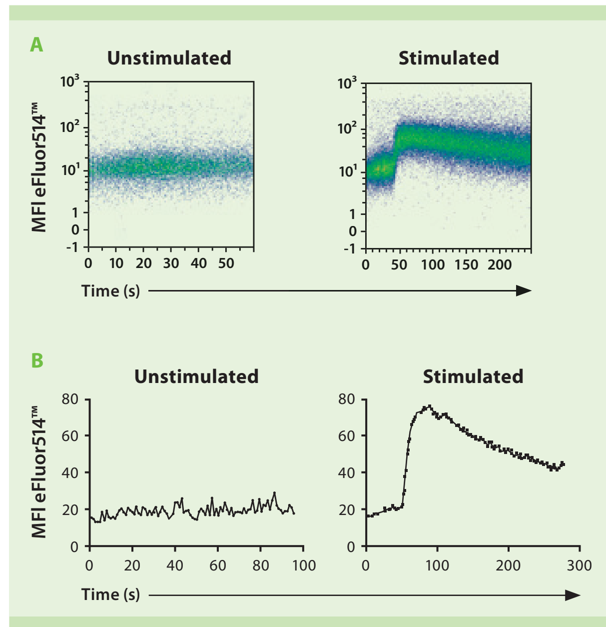


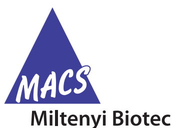
Figure 2: Ca^{2+} flux assay of Jurkat cells stimulated with ionomycin. (A) Representative dot plots and (B) kinetic plots show a rapid increase in fluorescence intensity upon Ca^{2+} influx.

Conclusions

The MACSQuant® X Analyzer facilitates Ca^{2+} flux assays and enables continuous data acquisition for reliable and reproducible results.

References

1. Berridge M. J. *et al.*, (2000) The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol. 1(1): 11–21.
2. Schwaller, B. (2012) The regulation of a cell's Ca^{2+} signaling toolkit: the Ca^{2+} homeostasome. Adv Exp Med Biol. 740: 1–25.
3. Paredes, R. M. *et al.*, (2008) Chemical calcium indicators. Methods. 46(3): 143–151.



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