METHOD FOR LOW CELL NUMBER SAMPLE PREPARATION – AUTOMATED UP-CONCENTRATION, WASHING AND STAINING

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Introduction

Manual pipetting and centrifugation-based washing can dramatically decrease cell recovery and viability, further lowering the cell number in already scarce samples. Therefore, sample preparation involving these conventional techniques in protocols for e.g. staining and washing of cells can be challenging.

The AcouTrap core technology uses non-contact acoustic trapping to capture cells in a microfluidic flow-through format, and acoustic-induced mixing to enhance binding kinetics, thus decreasing incubation times. AcouSort has developed novel methods for its AcouTrap system to enable handling of scarce cell samples and low sample volumes with high recovery.

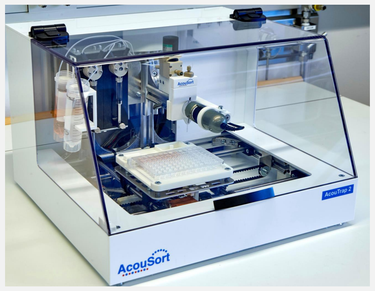

Figure 1: AcouTrap.

Experimental

Cell washing and staining using the AcouTrap (Fig. 1) was performed on cultured Jurkat cells. In the cell washing protocol, samples of 45,000 cells in ranging concentrations of 0.25-1 million cells/mL were aspirated and captured in the acoustic trapping unit (Fig. 2). While trapped, the cells were washed with PBS and subsequentially released in 200 µL. To perform in-trap cell staining (Fig. 3), fluorescent antibody dyes were aspirated over the cluster of trapped cells, followed by a wash. Cell samples were released in 200 µL and analyzed in a flow cytometer.

Figure 2: Acoustic trapping unit.

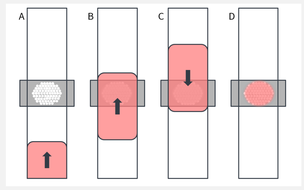

Figure 3: Schematic illustration of cell sample staining performed on a cell cluster in the acoustic trapping unit.

Results

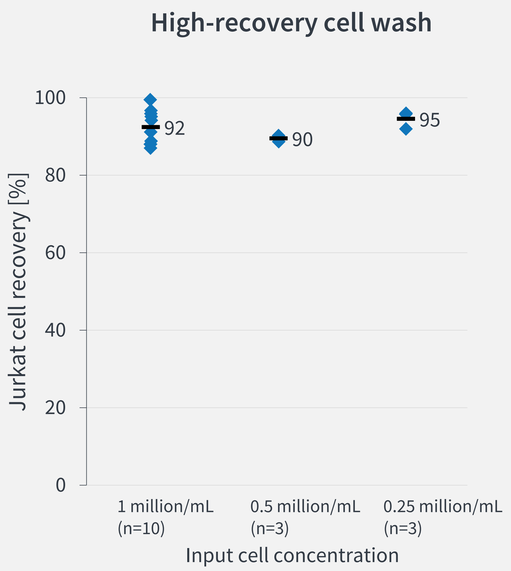

Figure 4: Recovery of 45,000 cells (in ranging input concentrations of 0.25-1 million cells/mL) with AcouTrap washing.

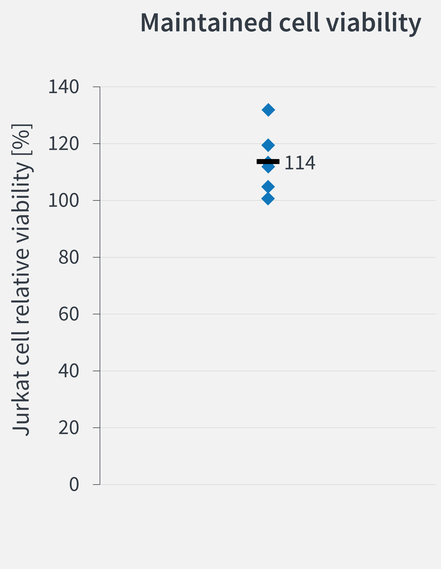

Figure 5: Relative cell viability of Jurkat cells after AcouTrap washing (PI negative cells [%] in output sample compared to input sample, n=6).

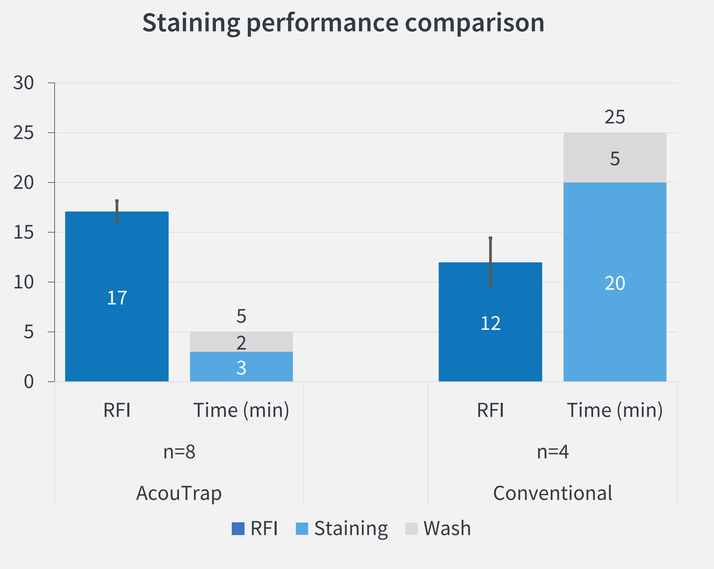

Figure 6: Comparison of relative fluorescence intensity (RFI), calculated as the median fluorescence intensity of the CD45 signal over the median fluorescence intensity of the isotype control, and processing time for staining and washing of Jurkat cells performed either using AcouTrap or a conventional protocol.

Conclusions

The AcouTrap is a unique tool for automated processing for up-concentration, staining and washing, combined or as individual protocols, of low cell number samples.

- Allowing for up-concentration to 1.5 million cells/mL in 30 µL
- Cell wash with high recovery ≥90%
- No loss of viability
- Integrated staining and washing produces samples with higher RFI than conventional protocol in a fraction of the time
- With customized acoustic trapping units, the throughput and capacity can be increased

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