Standardization of whole blood immunophenotyping using an automated staining protocol and recombinant antibodies

Antibody

Michael Sturges (michaelstu@miltenyi.com), Miltenyi Biotec, **Johannes Fleischer**, Miltenyi Biotec B.V. & Co. KG, **Kalpana Singh**, Miltenyi Biotec B.V. & Co. KG, **Friederike Sutorma**, Miltenyi Biotec B.V. & Co. KG, **Martin Büscher**, Miltenyi Biotec B.V. & Co. KG

Monitoring the state of the immune system by flow cytometry is a routine assay in both clinical and research settings. Although human whole blood samples are widely used for these immunophenotyping assays, they represent a critical source of variability and can impede standardization efforts. In general, the analysis includes two steps: staining of cell type–specific markers and lysis of erythrocytes. These steps are influenced by multiple parameters like incubation time, temperature, sample handling by different operators, and choice of reagents. All these parameters can introduce variability in analysis and diminish the overall reproducibility.

In this study, we show that automation of erythrocyte lysis and cell staining not only provides the basis for standardization, but additionally decreases hands-on time. We established an automated lysis and staining protocol on the MACSQuant® Analyzer 10 and verified its

proper function with an immunophenotyping panel. Automation not only includes lysis and staining: Preselected instrument settings and analysis templates are automatically applied, which simplifies cell analysis considerably.

We also designed an immunophenotyping panel based on recombinantly generated REAfinity™ Antibodies. They are superior to traditional hybridoma antibodies for several reasons: REAfinity Antibodies are recombinantly generated, thus ensuring higher lot-to-lot consistency. Additionally, they are mutated at the Fc region to abolish any background binding to FcγRs and therefore do not require addition of FcR blocking reagent during staining.