Measuring Real-Time Antibody Binding Kinetics for Quantification of Antibody-Binding Capacity

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Quantification of antibody binding capacity (ABC) allows the determination of antigen expression, which can be important in the diagnosis and monitoring of disease. Traditionally, the number of expressed antigens on the cell surface is estimated by measuring the fluorescence intensity of labeled antibodies bound to cells under equilibrium conditions. The amount of bound antibodies is then determined using calibration beads such as Sphero™ Rainbow Calibration Particles or Quantum™ Simply Cellular® Beads. However, these quantification methods can be confounded by several factors, including differences in fluorescence emission spectra and buffer/matrix effects that affect fluorescence yield. An alternative quantification method is based on antibody binding kinetics first demonstrated by Moskalensky *et al.* 2015 (DOI: 10.1016/j.jim.2015.11.002) and Khalo *et al*. 2018 (DOI: 10.1002/cyto.a.23494).
The kinetics-based method is independent of absolute fluorescence emission intensity and may be more robust than the calibration-based approaches. However, the protocols proposed in these publications involve several different preparations and measurements for 3 different antibody concentrations and 6 different time points for each antibody concentration. We have developed a method that measures antibody binding kinetics in real time, requiring only one measurement for each of the antibody concentrations. We present CD4 expression data of T lymphocytes for CD-Chex Plus® control samples in order to demonstrate the efficacy of our real-time kinetic measurement method and data analysis. Furthermore, we compare the determined ABC with the ABC determined by calibration with Quantum™ Simply Cellular® beads at equilibrium conditions.