

Reverse-Phase Protein Arrays

Antigen-Antibody Measurement by End-point and Kinetics (Rate)

Summary

Purified rabbit immunoglobulin G (RIgG) is spotted in protein microarrays, and the concentration of different concentrations of Goat Anti-Rabbit Gamma Globulin (GARGG) is measured by both extent (end-point) and rate (kinetics) of the Ag-Ab binding reaction during the same assay at specified incubation times. This demonstrates assay flexibility for reverse-phase protein arrays and antibody characterization by label-free immunoassay and either end-point or rate reaction.

Background

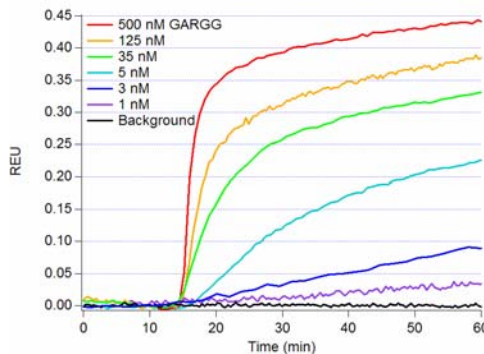
In forward-phase immunoassays, the goal is to immobilize “capture” antibodies with a very high affinity for molecules of interest (analytes) which are in solution. The reverse-phase immunoassay is generally more difficult since the goal is to immobilize the target molecule of interest (antigen) in such a way that antibodies in a sample such as in serum or as an antibody probe still have a high degree of affinity for that particular antigen. Often, care is needed to immobilize certain antigens so that the conformational shape of the molecule is in tact and the immunogenic portion of the molecule is exposed. Reverse-phase immunoassays are used in commercial diagnostics for autoimmune disease, infectious and allergic diseases, and in research for biomarker analyses using protein arrays.

The data here shows the quantitative measurement of Goat Anti-Rabbit IgG (GARGG) binding to rabbit IgG spotted on regular glass substrates. In this case, the RIgG surface antibodies are not capture antibodies but surface bound protein antigens, making this a reverse-phase assay. Using LFIRE™ and observation of the association kinetics of the binding reaction enables determination of analyte concentration by with rate reaction or end-point.

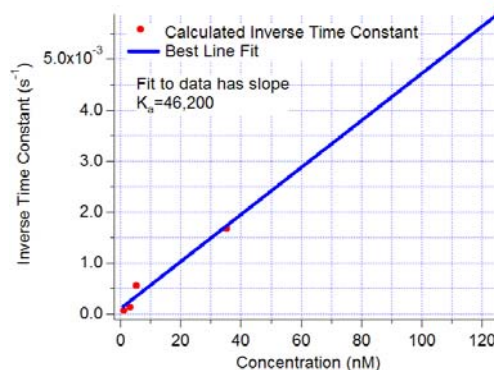
Method and Protocol

Microarray spots of purified RIgG were spotted on low sensitivity LFIRE™ slides at concentrations of 0.25 mg/ml and allowed to dry. Goat antibodies to Rabbit IgG (GARGG) were introduced at various concentrations from 1 nM to 500 nM and flow rates of 25 µl/min in a flow cell.

Results



The graph above shows the kinetic curves of reaction for the various concentrations of GARGG added. From this data, the time constants are determined and theoretically, the plot of the inverse time constants versus concentration yields a straight line as long as the interaction follows a simple model of molecular binding. This is represented in the second graph where the slope gives us the association constant, $k_a \sim 46,200 \text{ M}^{-1} \text{ s}^{-1}$, within 5% of published values. Using standard off-the-shelf slides, without our proprietary LFIRE™ substrates, we demonstrate the flexible utility of LFIRE for reproducible quantitative determination of protein analytes on multiple slide surfaces. In this case, our REU (relative ellipsometry unit) values are significantly below those obtained with our high sensitivity LFIRE™ slides, yet reliable quantitative binding results are obtained.



For the experiment above, the CV from spot to spot within the array was less than 4%, in line with high precision immunoassays.