Reichert Surface Plasmon Resonance (SPR) SR7500DC Dual-Channel System Application Note 9

Determining the Concentration of an Antigen Sample Using a Reichert SR7500DC

Introduction

The Concentration of an analyte can be determined directly using SPR. In this example, Anti-Human Serum Albumin Mouse Monoclonal (Anti-HSA) is immobilized directly to the sensor surface at a high level (greater than 1500 micro RIU). A range of Antigen concentrations (Human Serum Albumin or HSA) are injected over the surface that are expected to encompass the concentration in the unknown sample(s). The high surface concentration of Antibody creates a diffusion controlled binding response where the initial response is proportional to the concentration of the injected antigen analyte. In this example, the response at 60 seconds is plotted against the concentration of injected HSA to create a standard curve so that the concentration of HSA in unknown samples can be calculated. Known control samples are also injected.

Experimental

The experimental conditions are summarized in the following Table:

Ligand	Analyte	Analyte Concentrations	Association Time	Dissociation Time	Regeneration
Anti-HSA	HSA	40, 20, 10, 5, 2.5, 1.25, 0.626, 0.3125, 0.1562, 0.0781, 0.0391 and 0.0195 nM	3 min	3 min	10 mM Glycine pH 2.0 with 10% Glycerol

Results

The SR7500DC monitors this antibody-antigen interaction in real-time with simultaneous measurements of sample and reference channels. Figure 1 presents normalized, reference subtracted data from a series of 12 HSA injections at concentrations ranging from 0.02 to 40 nM. Between each injection the surface was regenerated with 10 mM glycine with 10% glycerol.

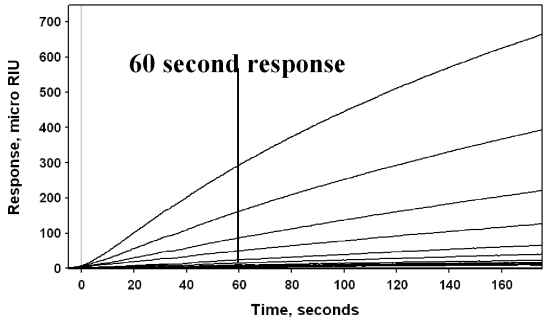

Figure 1: Normalized response versus time plots of HSA binding to surface immobilized Anti-HSA.

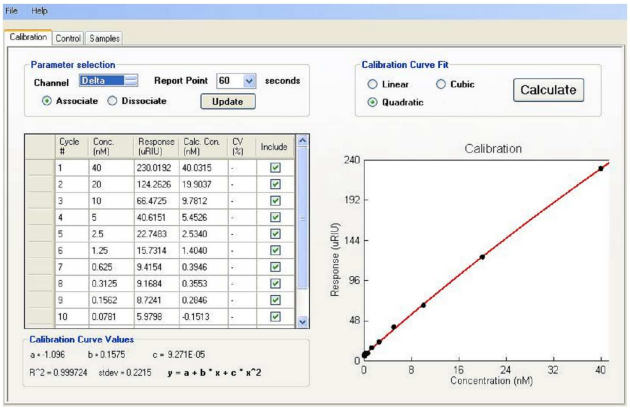

Figure 2: Plot of response at 60 seconds for HSA standard injections versus concentration of HSA.

Figure 2 shows the instrument response at 60 seconds vs. HSA concentration which is best fit to a quadratic. This plot can be used as a calibration curve to determine the active concentration of unknown samples. The SPR assay is a rapid direct measurement, independant of turbidity or color and does not require the use of labels or tags..

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