bioanalytics
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Application Note 09

Concentration Analysis

Determining the Concentration of an Antigen Sample Using the SR7500DC Dual Channel Surface Plasmon Resonance (SPR) System

The concentration of an analyte can be determined directly using SPR. In this example, anti-human serum albumin mouse monoclonal (Anti-HSA) was immobilized directly to the XanTec CMD500m dextran sensor chip at a high level (greater than 1500 micro RIU). A range of antigen concentrations (human serum albumin or HSA) was injected over the surface and was expected to encompass the concentration in the unknown samples. The high surface concentration of antibody created a diffusion controlled binding response where the initial response was proportional to the concentration of the injected antigen analyte. In this example, the response at 90 seconds was plotted against the concentration of injected HSA creating a standard curve so that the concentration of HSA in unknown samples could be calculated. Known control samples were also injected.

Experimental

The experimental conditions are summarized in the following table:

- Ligand: Anti-HSA
- Analyte: HSA
- Analyte Concentrations: 40, 20, 10, 5, 2.5,1.25, 0.626, 0.3125, 0.1562, 0.781, 0.0391, 0.0195nM
- Association Time: 3 min
- Dissociation Time: 3 min
- Regeneration: 10 mM Glycine pH 2 with 10% Glycerol

Results

The SR7500DC SPR system monitors this antibody-antigen interaction in real-time with simultaneous measurements of sample and reference channels.

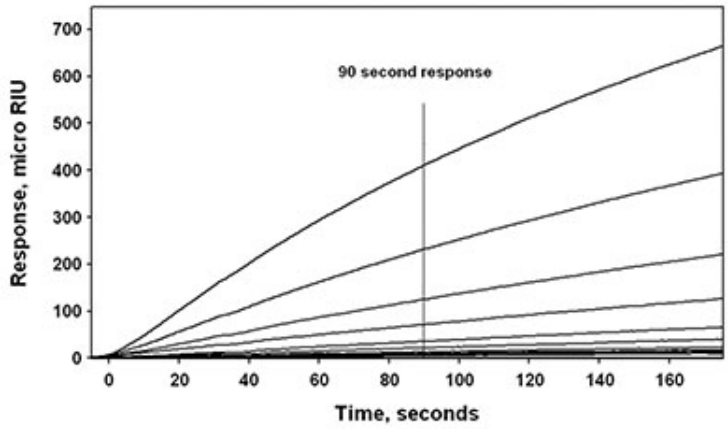

Figure 1 presents normalized reference subtracted data from a series of 12 HSA injections at concentrations ranging from 0.02 to 40 nM. Between each injection the surface was regenerated with 10 mM glycine with 10% glycerol. Illustrated here is normalized response versus time plots of HSA binding to surface immobilized Anti-HSA.

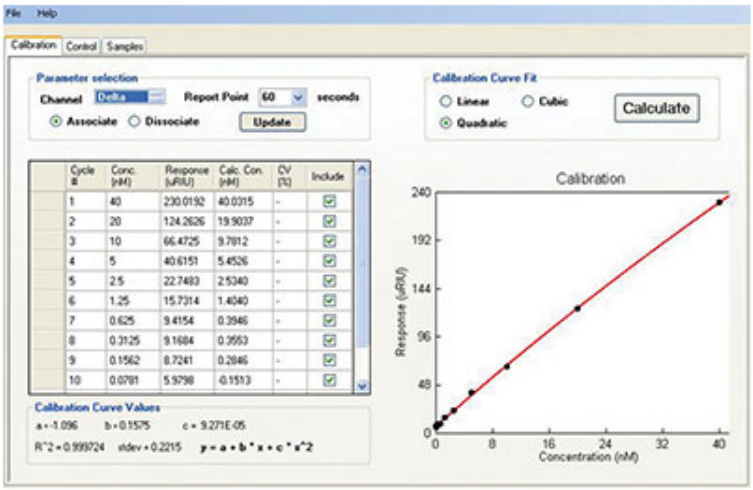

Figure 2 shows the instrument response at 90 seconds for HSA standard injections versus concentration of HSA which is best fit to a quadratic. This plot can be used as a calibration curve to determine the active concentration of unknown samples. The SPR assay is a rapid direct measurement, independant of turbidity or color and does not require the use of labels or tags.

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