



## Application Note 8

# Capture Surface Plasmon Resonance (SPR) Analysis Using a Reichert SR7500DC SPR System and Carboxymethyl Dextran Slides

There are several approaches that can be used for immobilizing ligands including covalent coupling (amine, thiol, etc.) and non-covalent capture experiments. For ligands that cannot withstand the lower pH needed for covalent coupling or that need to be attached in a more oriented manner, the capture approach is the preferred method. The capture experiment shown here is one where Goat Anti-Mouse IgG is amine coupled to a dextran (CMD500k) surface and then monoclonal Anti-HSA IgG is captured. Binding of Anti-HSA to human serum albumin (HSA) is followed over a series of concentrations. Results show excellent reproducibility for this multi-step experiment and good activity of the enzyme.

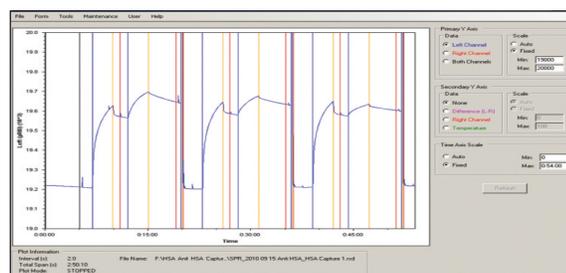
## Experimental

The experimental conditions are summarized in the following table:

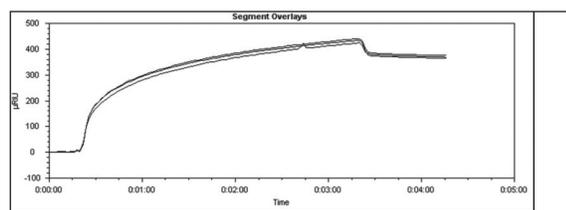
Ligand	Analyte	Analyte Concentrations	Association Time	Dissociation Time	Regeneration
Anti-HSA	HSA	1.25, 2.5, 5, 10 and 20nM	3 min	4 min	10 mM Glycine pH 2.0 with 10% Glycerol

## Results

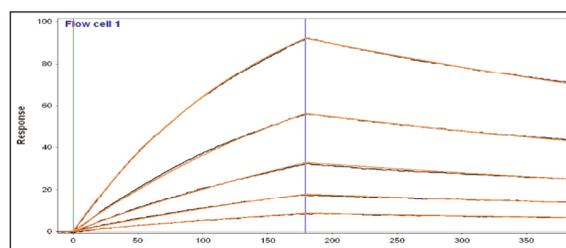
**Figure 1:** About 2,000  $\mu$ RIU of Goat Anti-Mouse IgG Fc was amine coupled to the CMD500k dextran surface. For each series of injections, a constant concentration of monoclonal Anti-HSA IgG (50  $\mu$ g/mL) was captured over the surface, and then varying concentrations of HSA were injected (see table). Both Anti-HSA and HSA were then removed during each regeneration cycle.



**Figure 2:** The good reproducibility of the capture step and the chemical stability of the CMD500k surface are seen here. Even after multiple injection regeneration cycles, the surface was stable and gave reproducible results.



**Figure 3:** The good reproducibility of replicate injections of HSA can be seen in this figure. HSA was injected at various concentrations (see table) and fit to a 1:1 binding model. The red lines are the fit obtained in Scrubber (Biologic Software). The equilibrium dissociation constant ( $K_D$ ) obtained was 4.93 nM.



[www.ReichertSPR.com](http://www.ReichertSPR.com)

Innovative precision instruments for over a century

### Corporate Headquarters

Reichert Technologies, Inc.  
3362 Walden Avenue  
Buffalo, New York 14043 USA  
Tel. +1 716-686-4500, Fax. +1 716-686-4555  
Toll Free USA 1-888-849-8955  
Life Sciences: +1 716-686-4522  
E-mail: reichertspr.lifesciences@ametek.com

### European Headquarters

Reichert Instruments GmbH  
Hubertusstrasse 2  
D-82229 Seefeld, Germany  
Tel. +49 8152 99 3530  
Fax. +49 8152 99 35311