

Binding Kinetics of an Antibody Serum Sample Using OpenSPR

Summary

- Kinetic binding of mouse immunoglobulin G (MIgG) to anti-MIgG from diluted whole antiserum was measured using the OpenSPR-XT™ instrument.
- The K_D was determined to be 145 pM, which is comparable to the purified control sample.
- OpenSPR™ can be used to accurately detect and measure the kinetics of analytes from crude samples.

Overview

A common application of the SPR technique is antibody screening, which is critical to the development of vaccines and other immunotherapies. Since this development often involves screening animal sera, analytes for these applications are often found in complex matrices. In this application note, OpenSPR was used to detect, and measure the kinetics of anti-MIgG from diluted antiserum. OpenSPR™ is an affordable and user-friendly SPR instrument that enables measurement of label-free binding kinetics of diverse biomolecular interactions. Additionally, positive and negative controls were used to confirm the activity and specificity of the immobilized ligand, and to verify the binding constants for the serum sample.

Materials and Equipment

- OpenSPR-XT™ Instrument
- OpenSPR™ Carboxyl Sensor
- OpenSPR™ Amine Coupling Kit
- TraceDrawer™ Kinetic Analysis Software
- Ligand: Mouse Immunoglobulin G (MIgG)
- Analyte: Goat Anti-mouse IgG, whole Antiserum
- Negative control: Human Immunoglobulin G (HIgG)
- Positive control: Purified Polyclonal Goat Anti-mouse IgG
- Blocking agent: Bovine Serum Albumin
- Running buffer: PBS-T (0.05% Tween-20), pH 7.4

Procedure

1. Following the start-up procedure in the software, the OpenSPR-XT instrument was set up, using PBS-T as the running buffer.
2. A Carboxyl Sensor was prepared following the ligand immobilization wizard, starting with a wash of 10 mM HCl, at a flow rate of 150 μ L/min, in both channels.
3. EDC/NHS solution from the OpenSPR Amine Coupling Kit was used to activate the carboxyl surface at a flow rate of 20 μ L/min, in both channels.
4. MIgG was immobilized on the sensor surface at a flow rate of 10 μ L/min in channel 2 only (designated as the sensing channel).
5. Bovine Serum Albumin (BSA) was injected at a flow rate of 10 μ L/min into both channels to block the reference channel and to help prevent non-specific binding.
6. Blocking solution from the OpenSPR Amine Coupling Kit was used to deactivate any unreacted carboxyl groups on the sensor.
7. HIgG (negative control) was diluted in the running buffer to a concentration of 400 nM and further diluted in 5-fold series.
8. Purified anti-MIgG (positive control) was diluted in the running buffer to a concentration of 400 nM and further diluted in 5-fold series.
9. Goat serum containing the anti-MIgG was diluted 50-fold (600 nM MIgG concentration), and further diluted in 5-fold series.
10. Each analyte sample was analyzed in order of low to high concentration at 50 μ L/min with an association period of 180 seconds, and dissociation period of 750 seconds.
11. The sensor surface was regenerated between each analyte injection with 2 subsequent pH 1.5 Glycine-HCl injections at 100 μ L/min.
12. Binding kinetics were measured using the TraceDrawer™ Analysis Software using a one-to-one fit model.

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Results and Discussion

The immobilization of MIgG on the OpenSPR™ Carboxyl Sensor is shown in Figure 1 with over 3500 RU of immobilization.

Figure 2 shows the binding and kinetic fit of anti-MIgG from diluted whole antiserum to the immobilized MIgG at 5 different concentrations. The data was fit to a one-to-one binding model in Tracedrawer. The calculated kinetic constants are presented in Table 1.

Figures 2 and 3 show the negative and positive controls respectively. The negative control (HIgG) shows no binding, while the positive control (purified anti-MIgG) bound as expected, and a kinetic fit was performed with a one-to-one binding model (Table 1).

The absence of binding with the negative control confirms the binding is specific. Furthermore, lack of interference of complex media on binding kinetics is shown by the comparability of the kinetic data for the purified antibody vs. whole serum. This demonstrates the OpenSPR's ability to measure kinetics for analytes in diluted crude samples. It should, however, be noted that since these are polyclonal antibodies, the kinetics represent an average value, not a precise binding constant. Accurate kinetic screening requires a homogeneous ligand (e.g. monoclonal antibody) and knowledge of the titer of the analyte antibody.

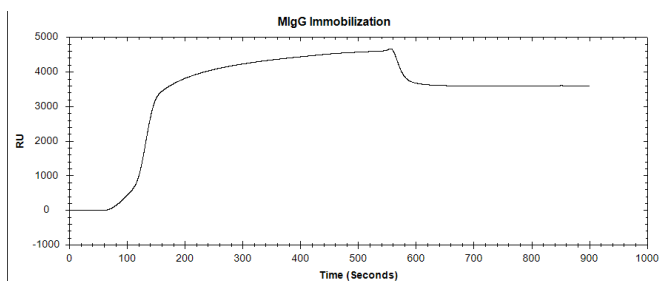


Figure 1. Ligand immobilization of MIgG to the OpenSPR™ Carboxyl Sensor.

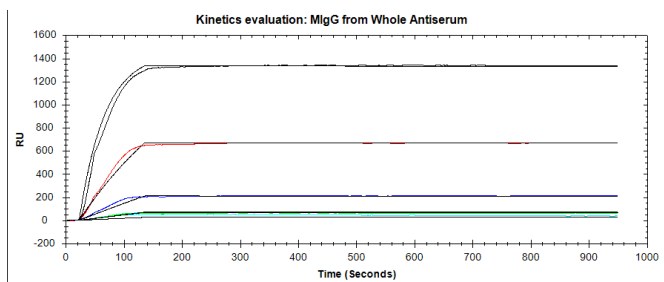


Figure 2. Binding of anti-MIgG at 600 nM, 120 nM, 24 nM, 4.8 nM, and 0.96 nM to the immobilized MIgG. The solid black lines represent the one-to-one kinetic model fits.

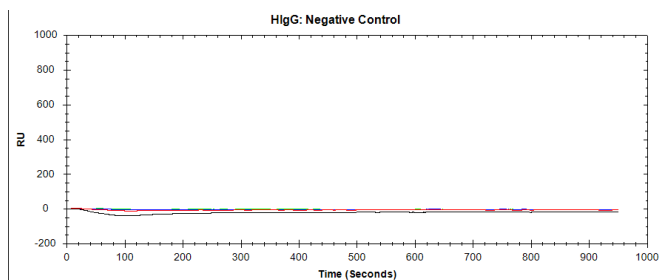


Figure 3. HIgG (negative control) injected over the immobilized ligand at concentrations of 400 nM, 80 nM, 16 nM, and 3.2 nM.

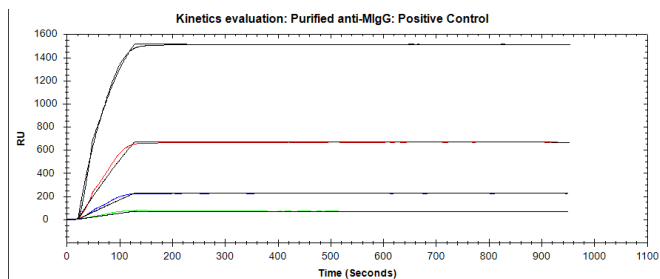


Figure 4: Binding of the purified MIgG (positive control) to the immobilized ligand at concentrations of 400 nM, 80 nM, 16 nM, and 3.2 nM. The solid black lines represent the one-to-one kinetic model fits.

Table 1. Kinetic values measured using OpenSPR™ data with the TraceDrawer™ analysis software

| | Whole Serum MIgG | Purified MIgG |
|------------------|-----------------------------|-----------------------------|
| k_{on} [1/M*s] | 3.77e4 ($\pm 3.39e0$) | 3.36e4 ($\pm 1.07e0$) |
| k_{off} [1/s] | 5.47e-6 ($\pm 3.82e-6$) | 4.94e-6 ($\pm 8.19e-6$) |
| K_D [M] | 1.45e-10 ($\pm 1.01e-10$) | 1.47e-10 ($\pm 2.44e-10$) |

Conclusions & Summary

This study demonstrates that the OpenSPR can be used to precisely detect and measure kinetics between an immobilized ligand and an analyte in a diluted complex medium. The positive and negative controls established the specificity and activity of the immobilized ligand.

The kinetic constants obtained for the MIgG from whole serum are consistent with the values obtained with the purified MIgG used as positive control. This supports the use of OpenSPR for applications such as antibody screening.

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