Application Note: Determination of alpha-lactalbumin concentration using OpenSPR™

Summary

- Anti-alpha-lactalbumin was immobilized onto a COOH sensor. An immobilization density of 3500pm was measured with excellent stability
- Kinetic binding of anti-alpha-lactalbumin to alpha-lactalbumin was measured using the OpenSPR instrument
- The assay's dynamic range is 15ng/ml – 1000ng/ml with a theoretical detection limit of 3ng/ml
- OpenSPR can be used as a concentration assay for determining the level of alpha-lactalbumin

Overview

Alpha-lactalbumin is a 14kDa protein present in all mammalian milk. It is the second most abundant whey protein in human milk, and is often added to infant formulae due to its high nutritional value. Therefore there is a need for a concentration assay that can determine supplement levels in infant formulae, natural levels in bovine milk, and pharmaceutical level in milk protein isolates.

SPR provides a simple, fast and cost-effective platform compared to techniques such as ELISA as it doesn't require labels, it obtains results in real time, and the surface can be used for many samples. In this application note, we develop an assay for measuring alpha-lactalbumin concentration using the OpenSPR platform.

Materials and Equipment

- OpenSPR Instrument
- OpenSPR COOH Sensor Chip
- OpenSPR Amine Coupling Kit
- TraceDrawer Kinetic Analysis Software
- Ligand: goat polyclonal anti-bovine alpha-lactalbumin immunoglobulin G (IgG) antibody
- Analyte: alpha-lactalbumin
- Running Buffer: HBS-EP
- Regeneration Buffer: Glycine-HCl pH 2.5

Procedure

1. Following the start-up procedure found in the OpenSPR manual, setup the OpenSPR instrument and software.
2. Load a COOH sensor chip into the OpenSPR instrument and pump running buffer until the baseline signal is stable.
3. Activate the surface with EDC/NHS in sodium acetate buffer at pH 5.5.
4. Dilute the anti-alpha-lactalbumin IgG to 200µg/ml into sodium acetate at pH 5.5.
5. Inject the alpha-lactalbumin at 20ul/min for a 5-minute incubation time with the activated surface.
6. Prepare dilutions of the analyte alpha-lactalbumin into the running buffer at concentrations of 3000, 2000, 1000, 750, 500, 300, 200, 100, 50, 10, and 1 ng/ml.
7. Inject each concentration for a 3-minute association time and a 6-minute dissociation time, performing a regeneration injection of glycine-HCl in between each concentation.
8. Import the data into TraceDrawer™ for analysis.

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Results and Discussion

The immobilization of the anti-alpha-lactalbumin on the COOH sensor chips produced an immobilization density of 3500pm with excellent stability. Following blocking of the surface with ethanolamine, two conditioning injections of 3000 ng/ml alpha-lactalbumin were performed with glycine-HCl regeneration in between. Following the conditioning the 11 different concentrations of alpha-lactalbumin were injected with regeneration in between each.

Once the experiment was completed, the data was imported into TraceDrawer™ and analyzed with the affinity/EC50 module to determine the response equation.

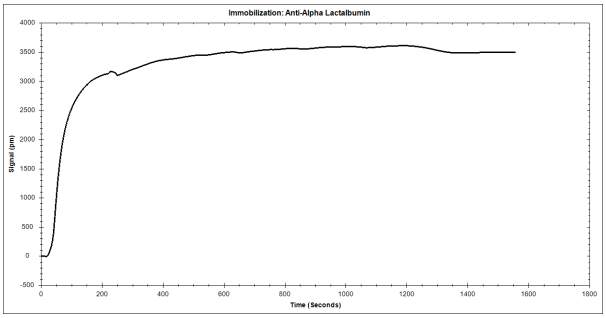

Figure 1. Ligand immobilization of alpha-lactalbumin onto COOH sensor chip.

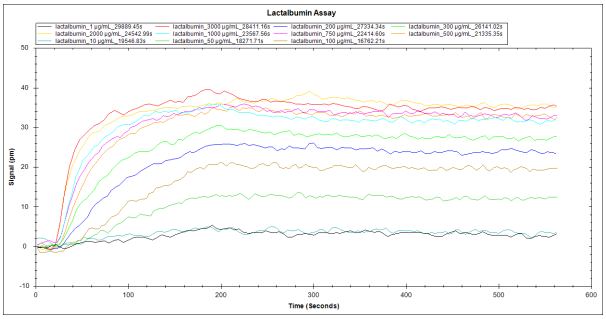

Figure 2. Binding of alpha-lactalbumin to anti-alpha-lactalbumin at concentrations of 3000, 2000, 1000, 750, 500, 300, 200, 100, 50, 10, and 1 ng/ml.

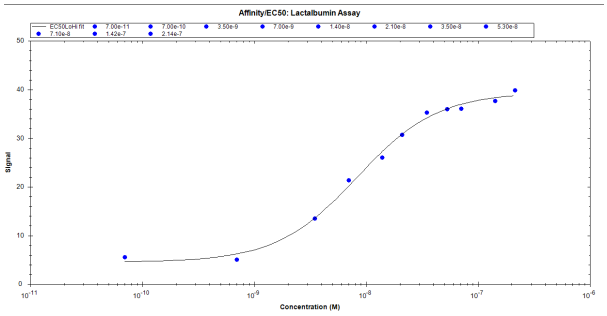

Figure 3. Affinity analysis of alpha-lactalbumin binding assay.

The response equation is of the following form:

$$Y = R_{max} * c/(c+K_D) + offset$$

Where Y is the SPR response, R_{max} is the signal at saturation, c is the analyte concentration and K_D is the affinity constant. Using non-linear regression, the following equation is determined for the assay:

K_D: 8.47nM

R_{max}: 37.2 pm

Offset: 3.65 pm

Y= 37.2*c/(c+8.47nM) + 3.65

This fit yields excellent errors on all constants as well as a low chi squared value of 1.55. Unknown concentrations of alpha-lactalbumin can be determined by inputting the SPR signal from the sample as "Y" and solving for the concentration.

The assay's dynamic range is 15ng/ml – 1000ng/ml with a theoretical detection limit of 3ng/ml. These parameters could be further improved or modified with additional assay optimization to fit different operational needs. The assay shows excellent repeatability with 6% CV between injections of the same concentration.

The performance of this assay is sufficient for use in industrial applications and compares well with the assay developed on a standard SPR system that this application note was based on[1].

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Conclusions and Summary

This study demonstrates how OpenSPR can be used as a concentration assay for determining the level of alpha-lactalbumin. The low capital cost and low cost per experiment, low maintenance, and ease of operation of the OpenSPR instrument makes it an excellent tool to replace ELISA assays in this and many other applications.

The automation of the OpenSPR-XT allows the unattended analysis of hundreds of samples per day without the need for an operator, further saving an organization time and cost. The assay can provide the results of an unknown sample in under 5 minutes and can be fully automated using OpenSPR-XT, providing an excellent alternative to traditional ELISA methods.

[1] H. Indyk et al., "The alpha-lactoglobulin content of bovine milk: Development and application of a biosensor immunoassay," International Dairy Journal, vol. 73, pp. 68-73, 2007.

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