Active Concentration Determination

QUICK FACTS

Range = 40 – 0.05 µg/ml

CV = ~3–6%

LOD = <0.08 µg/ml

Resolution = <0.02 µg/ml

OBJECTIVE

To determine the active concentration of myoglobin and mouse IgG respectively in samples, using the Attana A100® C-Fast system.

CONCLUSIONS

- Attana A100 is a useful tool for assessing active concentration and kinetics of antibodies and small proteins.
- Attana A100 can be used to follow protein purification, and monitor titer changes. It can also be used for QC testing batch-to-batch differences and to probe protein stability.
- The assay is automated, time saving and has a lower variability than ELISA.

BACKGROUND

When dealing with proteins, whether antibodies, recombinant proteins or biomarkers, it is essential to be able to measure active concentrations with great accuracy and specificity. Current standard methods do not capture the active concentration or isomer influence and require time consuming procedures. In this Application Example we demonstrate that the active concentration of proteins of different molecular weights can be measured in real time using the Attana A100 C-Fast system.

ATTANA A100 C-FAST BIOSENSOR

The Attana A100 C-Fast biosensor utilizes the Quartz Crystal Microbalance (QCM) technique for real time, label-free measurements of molecular interactions. When molecules are added to, or removed from the sensor surface, the change in the resonance frequency corresponds to the change in mass on the surface. By immobilizing a target molecule to the sensor surface, and flowing an interacting molecule over the surface, the interaction can be studied in real time. The real-time information can provide kinetic, affinity and specificity data on the interaction.

METHOD

The specificity-giving antibodies were immobilized on carboxyl surfaces at 50 µg/ml in HBST running buffer (10mM HEPES buffered Saline containing 0.005% Tween® 20) at a flow rate of 10 µl/min (**Table 1**). The myoglobin antigen was diluted to 40, 20, 5, 1, 0.5, 0.1, 0.05µg/ml and the IgG to 25, 5, 1, 0.5, 0.1, 0.05 µg/ml and subsequently injected over the surface.

The association phase slope was plotted against concentration to calculate a standard curve. Unknown samples were then injected, their slopes calculated, and their active concentrations determined. Quintuplicate injections were made to firmly determine measurement robustness.

RESULTS

Myoglobin Concentration assay: Reference injections using sample buffer without protein was used as negative control and produced a slope of 0.04 Hz/s. Specificity of binding was tested by injection of the unspecific control (BSA). As seen this did not result in a slope greater than the negative control despite a concentration of 10 µg/ml. CV values of the standard curve did not exceed what is shown for the unknown samples in **Table 2** and were typically lower. The data is modeled by a 2nd order equation producing an R-value of 1. It can also be concluded from the experiment that the resolution is at least 0.01 Hz/s, corresponding to <0.2 µg/ml. The level of detection for the myoglobin assay was determined to be 0.04 Hz/s.

Sample	Conc.	CV	Slope
Unknown A	1.2µg/ml	6%	0.19Hz/s
Unknown B	1.0µg/ml	6%	0.18Hz/s
Unknown C	0.5µg/ml	3%	0.13Hz/s
Negative Control	0µg/ml	15%	0.04Hz/s
Specificity Control	10µg/ml	14%	0.04Hz/s

Table 2: *Unknown myoglobin samples. Concentration of unknown myoglobin samples run in quintuplicate.*

Immobilized Ligand	Molecular Weight Ligand	Concentration Ligand	Instrument Response	Molecular Weight Analyte
mAb (anti-myo)	150kDa	50µg/ml	150Hz	17kDa
mAb(anti-mouse IgG)	150kDa	50µg/ml	250Hz	150kDa

Table 1: *Basic information on the interacting partners.*

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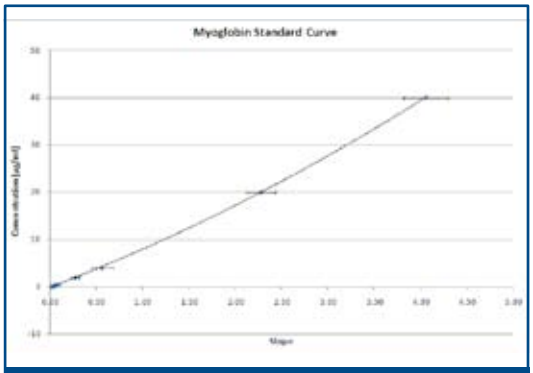

Figure 1: *Example of standard curve. Myoglobin standard curve fitted with a 2nd order equation, error bars are standard deviations.*

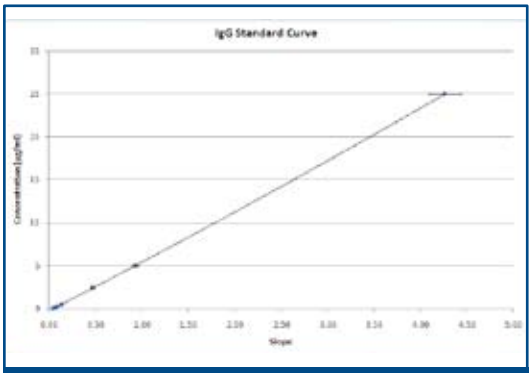

Figure 2: *Example of standard curve. IgG standard curve fitted with a 1st order equation, error bars are standard deviations.*

IgG concentration assay: Reference injections using sample buffer without protein was used as negative control and produced a slope of 0.03 Hz/s. Specificity of binding was tested by injection of the unspecific control (BSA). As seen, this did not result in a slope greater than the negative control despite a concentration of 10 µg/ml. CV values of the standard curve did not exceed what is shown for the unknown samples in **Table 2** and were typically lower. The data is modeled by a 1st order equation producing an R-value of 1. The level of detection for the IgG assay was determined to be 0.04 Hz/s.

Attana Materials Used	Item Code
Attana Carboxyl Sensor Chip	3616-3033 *(pack of 3)* 3616-3103 *(pack of 10)*
Amine Coupling Kit	3501-3001
HBS-T 10X (250 ml)	3506-3001
C-Fast: 3.1	3420-3001
Attester™: 3.1	3410-3001
Attester™ Evaluation: 3.1	3430-3001

Attana AB
Björnnäsv. 21. SE-113 47 Stockholm, Sweden
Telephone: +46 8 410 200 00, E-mail: info@attana.com
www.attana.com

attana sensor technologies