

■ Quantification sensor for concentration of antibody drugs

Protein A is a protein having antibody affinities. This time, we developed a sensor quantitating for concentration of antibody drugs using this behavior. Using a sensor with protein A bound on the surface reacted with anti-CRP, repeated measurement was made. The results showed a possibility of measurement at varieties of concentration and good variation level after the repeated measurement. Multiple data acquisition with high reliability can be achieved with one protein A sensor.

The above sensor was developed with a cooperation by Immune Biological Laboratories Co., Ltd.

● 1. Protocol

- Sensor: 30MHz twin sensor (with protein A immobilized)
- Flow rate: 20 μ L/min
- Sample amount: 100 μ L
- Running buffer: PBS
- Sample: Anti CRP antibody (Rabbit IgG)
- Regenerating solution: 10mM glycine -HCl

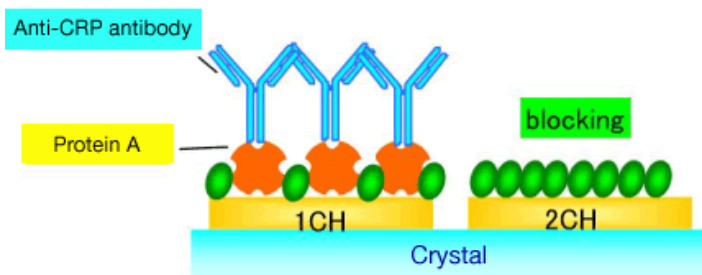


Fig.1 Diagram

● 2. Reaction waveform

After anti-CRP antibody injection, regenerating solution was injected. The following waveform was acquired after 3 times of the regenerating solution injection. The waveform shows that the base line returned to the original position after the regeneration and the antibody reaction amount level was almost kept at each measurement. Additionally, Reaction electrode waveform was overlapped to differential waveform.

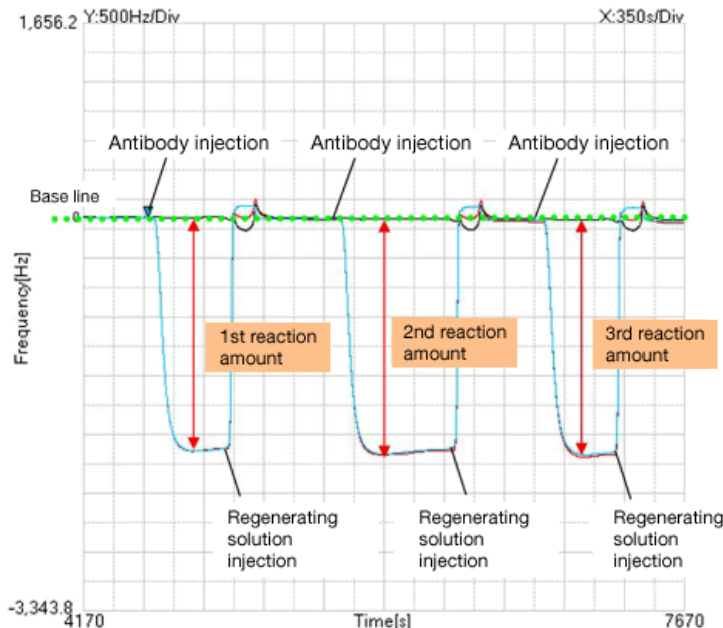


Fig2: Measurement waveform

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● **3. Concentration dependency**

The measurement was made at 500ng/mL, 1 μg/mL, 5 μg/mL and 10 μg/mL of the anti-CRP antibody concentration, and then the standard curve was made. This shows that quantitative measurement between 500ng and 10 μg/mL concentration is possible.

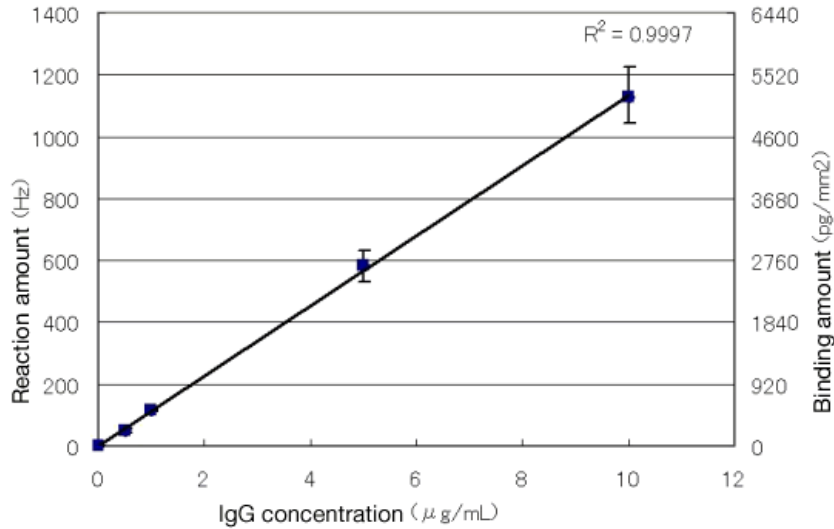


Fig.3:Standard curve of antibody measured by protein A sensor

● **4. Validation for variation of values conducted one sensor**

1 μg/mL anti-CRP antibody was measured 8 times with one protein A sensor.

The result showed that CV value (used as barometer of variation) was good ,specifically 5%. This also shows that the repeated measurement is possible enough.

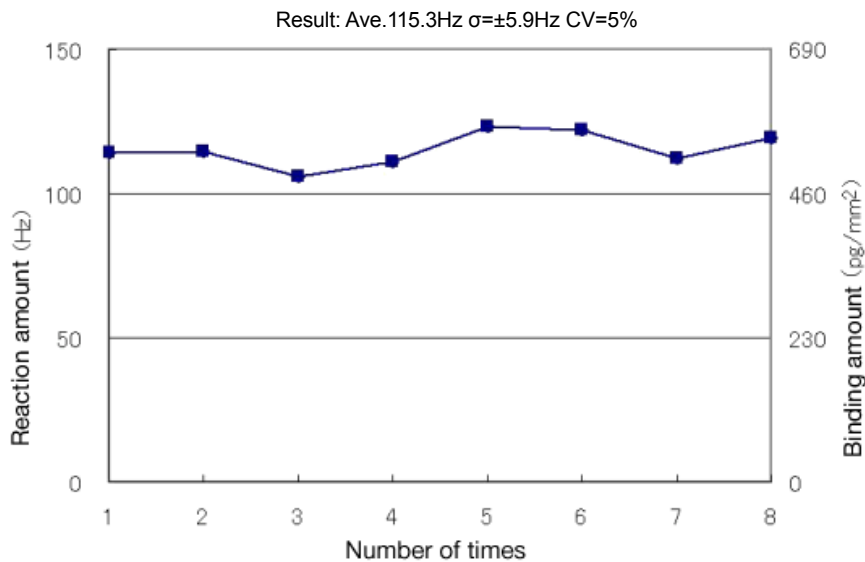


Fig.4:Result for variation of values used by one sensor with repeated measurements at antibody 1 μg/mL

● **5.Glossary**

- Protein A : A kind of staphylococcus aureus cell-wall protein. This substance binds to antibody specifically, and so is used widely for purification of antibody.
- CV value : This stands for coefficient of variation, and is used as barometer of variation.