

■ Measurement of label free immune response of the acute phase protein CRP (C reactive protein)

CRP and Antigen-Antibody reaction were measured after immobilizing Anti CRP IgG on Au electrode and blocking it with 0.1% BSA solution.

● 1. Protocol

After anti CRP IgG was immobilized on an Au electrode, it was blocked with 0.1% BSA solution. Next, CRP was injected and the antigen-antibody reaction was observed.

Please see figure 1. The measurement conditions are:

- Sensor: 30MHz twin sensor
- Sample amount: 100 μ L
- Running buffer: PBS
- Antibody: 100 μ g/mL Anti CRP IgG
- Blocking: 0.1% BSA
- Antigen: CRP

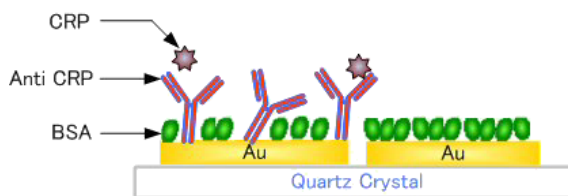


Fig.1 Diagram

● 2. Reaction waveform

Figure 2 shows the reaction monitoring. This wave form was gotten with CRP (antigen) 10 μ g/ml, Flow rate: 50 μ L/min.

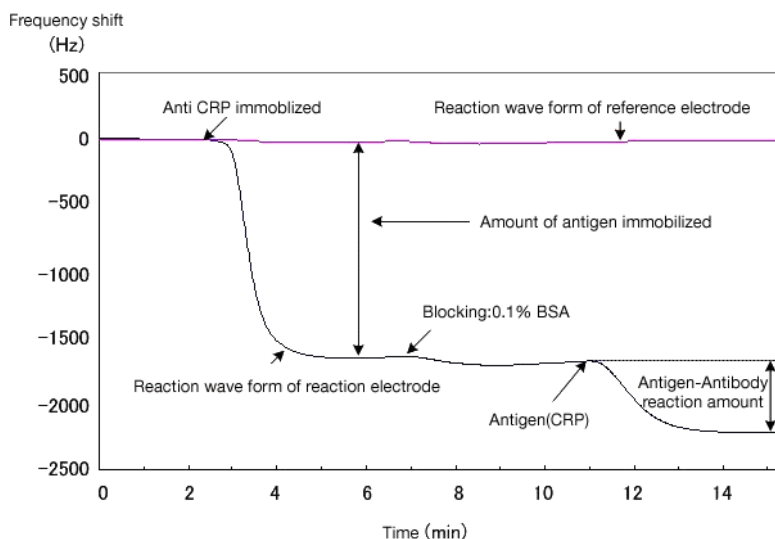


Fig.2. Monitoring of CRP antibody-antigen reaction

● 3. Concentration dependency

Figure 3 "Standard curve" shows the measurement results (Concentrations: 10ng/mL, 100ng/mL, 1 μ g/mL, 10 μ g/mL, N=2). The results show that CRP can be measured quantitatively in the concentration range between 10ng/mL and 10 μ g/mL.

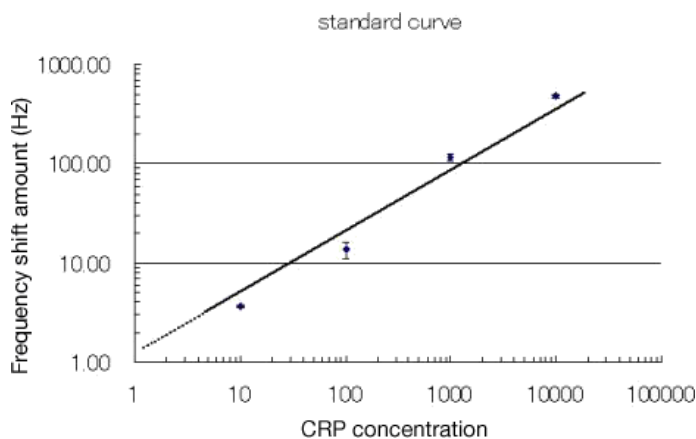


Fig.3. Standard curve

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● 4. Concentration & reaction responsiveness

Figure 4 "concentration-responsiveness relationship" shows the temperature and reaction responsiveness. This was obtained when the antigen-antibody reaction occurred (Flow rate for IgG antigen-antibody = 50 μ L/min). NAPiCOS enables not only the real time measurement of the reaction speed but also the analysis of the reaction responsiveness.

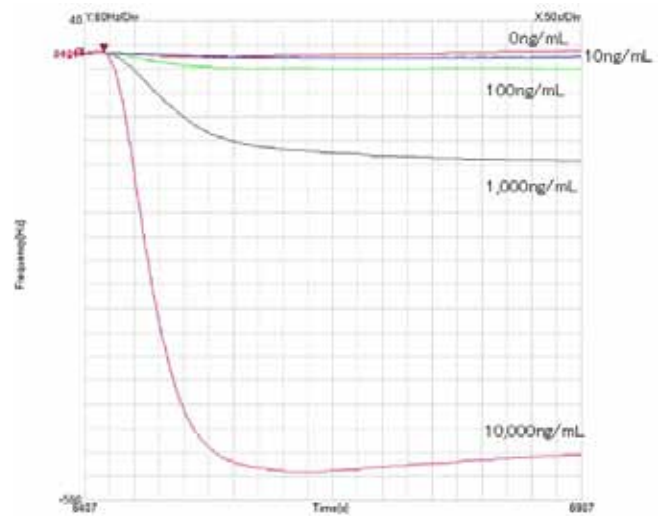


Fig.4: Concentration-responsiveness relationship

● 5. Antibody immobilization

The antibody was immobilized on the gold electrode of the crystal sensor. Since the time for immobilization in flow channel was very short, membrane was formed as shown below by using the popularly used ELISA plate. Please feel free to contact us about the multi-plate to fix the crystal sensor. If research sample containing ethanol is used, this method is highly recommended. When repetitive processes such as the immobilization of antibody and blocking are needed, the processes from (1) to (6) shall be repeated.

(1) Batch plate



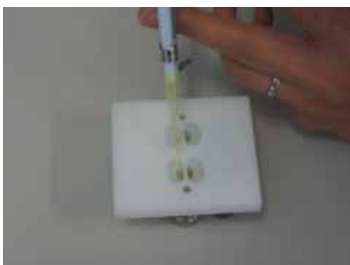
(2) Placing the batch plate on a sensor



(3) Electrode on the batch plate (top view)



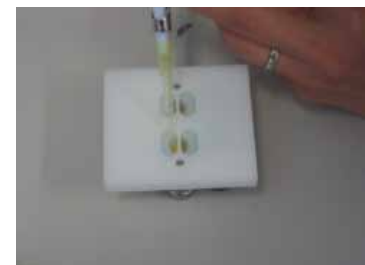
(4) Injecting and immobilizing the sample on the surface of the electrode



(5) Cleaning



(6) Removing the remaining liquid



● 6. Glossary

- CRP (C-Reactive protein): When there is tissue damage due to inflammation or cancer, C-Reactive protein (CRP) is produced and is released into the blood. Since CRP is sensitive to tissue damage, it is used as clinical marker for inflammation, cancer and so on.
- IgG (Immunoglobulin G) : A kind of immunoglobulin G. It is an antibody and has antigen binding property. In this experiment, Anti CRP IgG was used as antibody for CRP.