

■ Immuno-measurement of low-concentration insulin (100pg/mL ~ 20ng/mL)

A measurement of Insulin, a peptide hormone (MW 58,000), was performed through antigen-antibody reaction. The antibody labeled with colloidal gold was used as amplification reagent. The wave form showed that a reaction occurred only on the reaction electrode. This experiment was made with the cooperation of Morinaga Institute of Biological Science Inc. The insulin and antibody labeled with colloidal gold were provided by this institute.

● 1. Protocol

- Sensor: 30MHz twin sensor
- Flow rate: 50 μ L/min
- Sample amount: 100 μ L
- Running buffer: PBS
- Sample: Insulin
- Amplification reagent: antibody labeled with colloidal gold

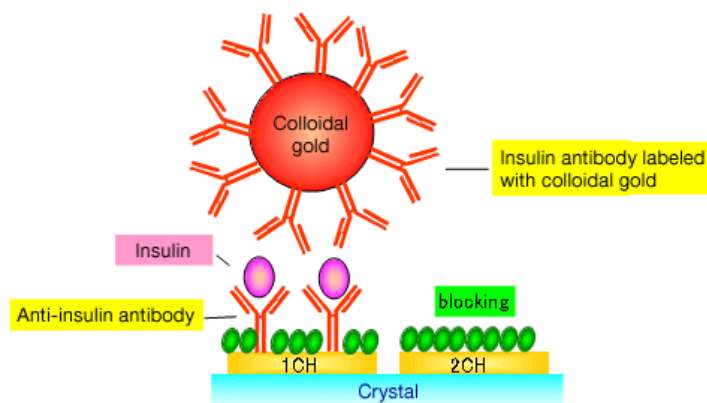


Fig.1 Diagram

● 2. Reaction waveform

(1) First of all, antibody was injected and then immobilized.

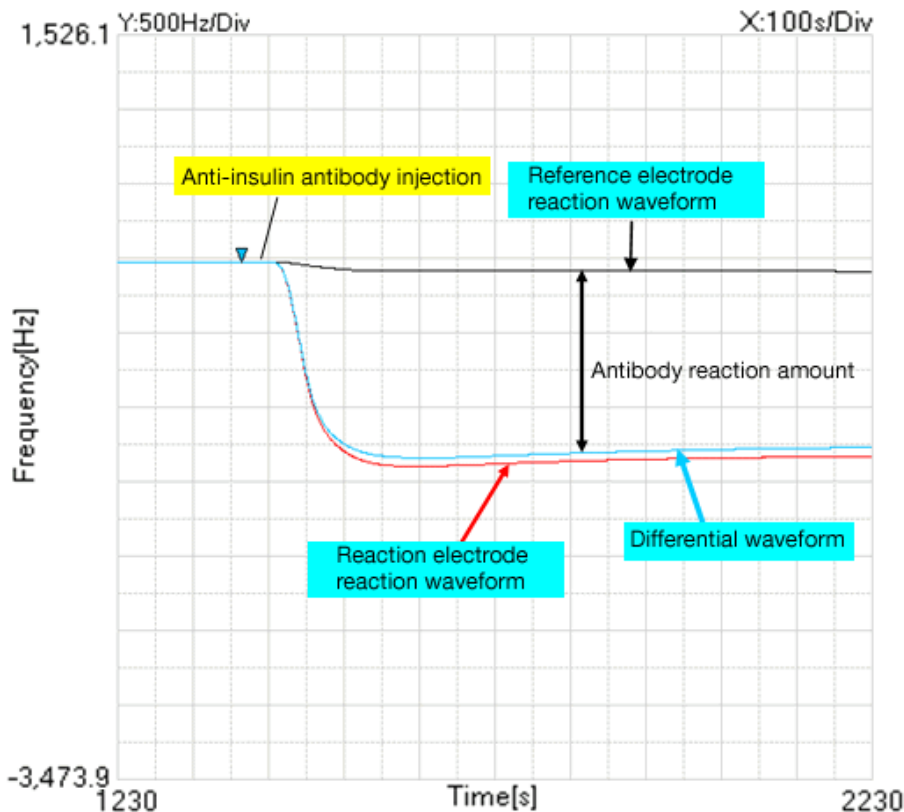


Fig.2: Waveform of antibody immobilized

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(2) Sample measurement & amplification

Next, insulin was injected, after which antibody labeled with colloidal gold was injected. This process was carried out at each insulin concentration. Figure 3 shows the overlaid reaction waveforms of each concentration.

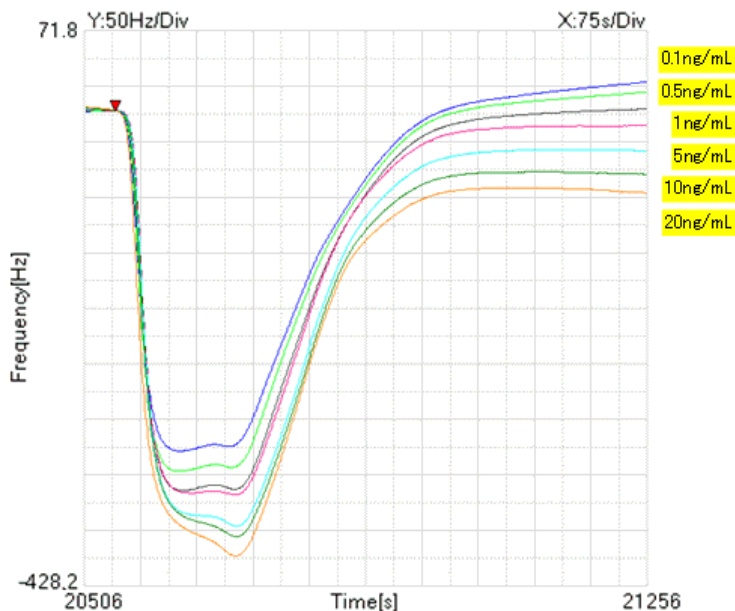


Fig.3 Reaction waveforms overlaid

● 3. Concentration dependency

Insulin was measured at concentrations 0.1 ng/mL, 0.5 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL and 20 ng/mL, and then standard curve was conducted with the values amplified (N=3). The reaction amount was cumulative, because continuous injections were made.

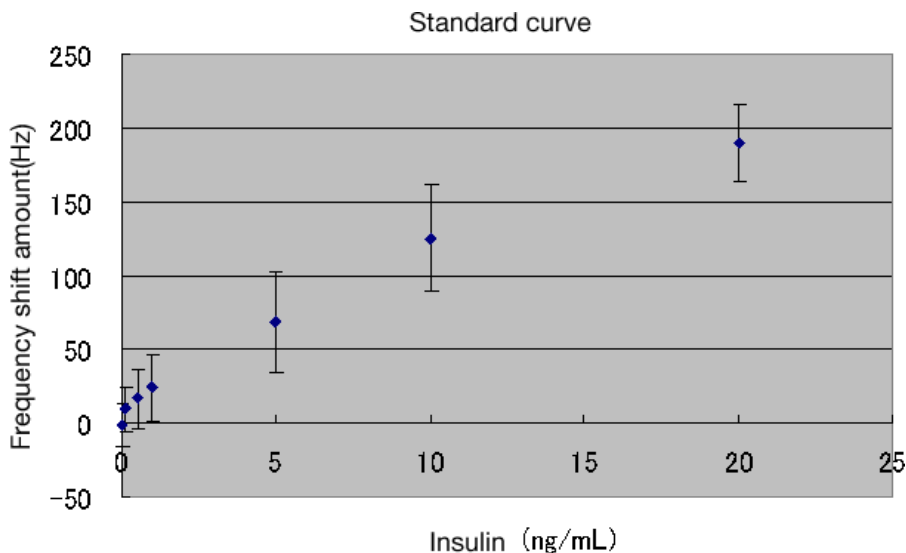


Fig.4: Standard curve after amplification

● 4. Glossary

- Insulin:** A kind of hormones secreted from pancreas. This stimulates to take sugar into cells, and so is involved in the sustention of blood glucose concentration level.
- Colloidal gold:** Colloid is a particle with 1-100nm diameters, and this is made when mixing with certain substance. Colloidal gold labeled antibody (antibody bound with colloidal gold surface) is of a nature as to become red due to aggregation of the gold when rebating to antigen. So, this is used is used for dyeing antigens. At this experiment, the great amplification using mass of the colloidal gold was confirmed, when sending the colloidal gold labeled antibody.