

■ Measurement of low-molecular substance

An example of low-molecular substance measurement. Low-molecular substance measurement can be replaced to high mass one measurement, using competitive inhibitory method. Thus, low-molecular substance biotin could be measured.

● 1. Protocol

- Sensor: 30MHz twin sensor
- Flow rate: 50 μ L/min
- Sample amount: 100 μ L
- Running buffer: PBS
- Sample: Biotin (Molecular Weight: 244)

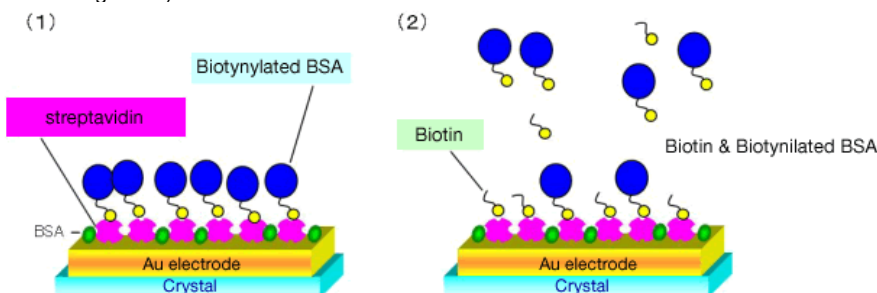


Fig.1: Diagram for biotin measurement (Direct competitive inhibitory method)

(1) Blank measurement

BSA bound with biotin (biotinylated BSA) was injected on the sensor coated with streptavidin. The reaction amount was defined as blank Value.

(2) Competitive inhibitory measurement

Biotinylated BSA, which was the same concentration as the above (1) and was mixed with biotin as single substance in advance, was injected. The biotin and the BSA bound with biotin competed each other, and binding amount of the latter one was decreased comparing to the above (1).

● 2. Reaction waveform

Blank waveform acquired after Biotinylated BSA injection is the figure 2-1, and waveform after Biotinylated BSA and biotin (1 μ g/mL) injection is the figure 2-2. Each solution contained 1% DMSO, and so the frequency shifted down once due to composition change. Then, each bound amount was acquired after changing to the running buffers. The waveforms show the shift down of the reaction amount, comparing to the blank waveform.

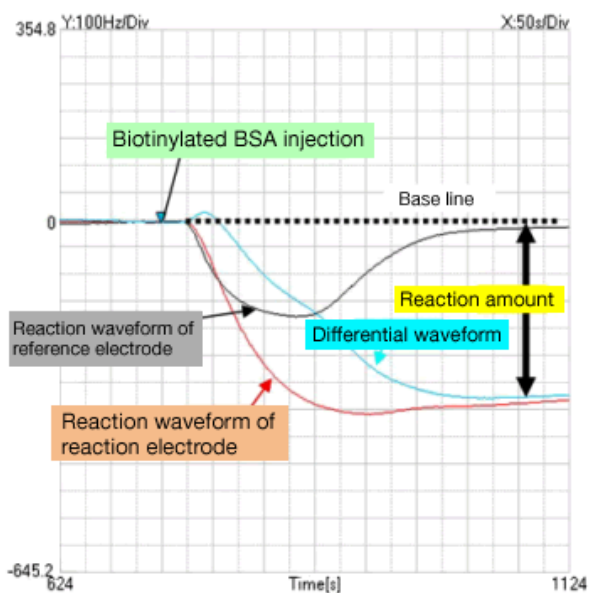


Fig.2-1: Blank waveform of Biotinylated BSA (10 μ g/mL, 1% DMSO)

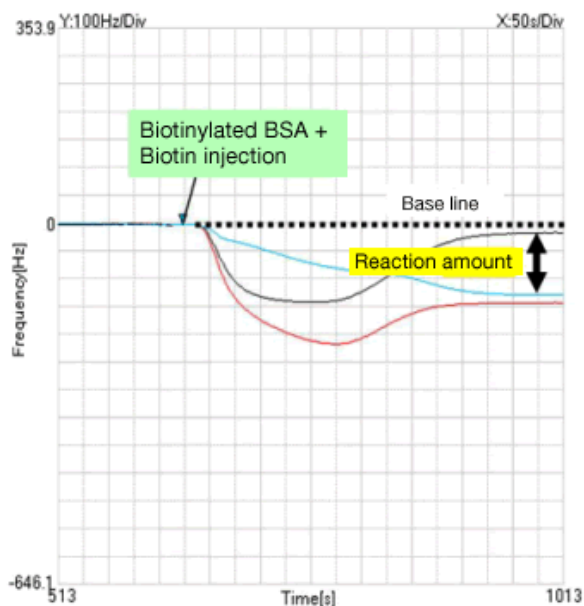


Fig.2-2 Biotin coexistence waveform
BSA bound with biotin 10 μ g/mL & Biotin (1% DMSO) 1 μ g/mL

Fig.2: Measurement waveform

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● 3. Concentration dependency

According to the table 1, the measurements with the competitive inhibitory method were made at the concentrations 1ng/mL, 10ng/mL, 100ng/mL, 1 μg/mL and 10 μg/mL. Then, Standard curve after Logit transformation was made.

Table 1: Concentration of each sample

Biotinylated BSA	Biotin	DMSO concentration
10 μg/mL	-	1%
10 μg/mL	1 ng/mL	1%
10 μg/mL	10 ng/mL	1%
10 μg/mL	100 ng/mL	1%
10 μg/mL	1 μg/mL	1%
10 μg/mL	10 μg/mL	1%

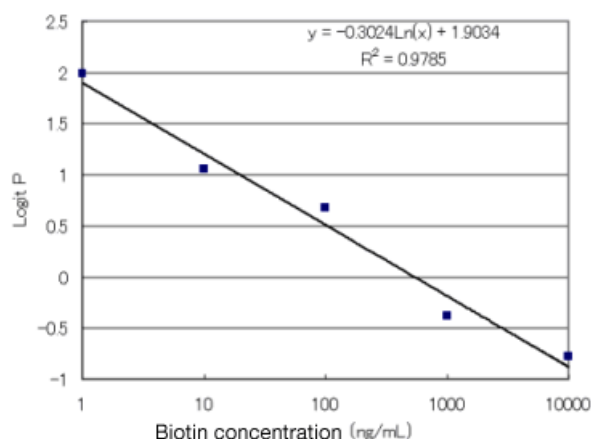
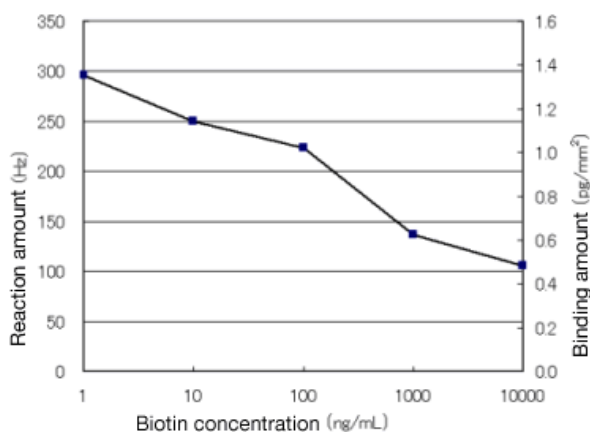


Fig.3: Standard curve of biotin

Reaction amount (Hz) and binding amount (pg/mm²) against biotin concentration

Standard curve after Logit transformation

● 4. Measurement of antibacterial agent X

The followings are actual measurement samples on antibacterial agent with Molecular Weight 300. The data shows that quantification with low concentration such as 10ng/mL is possible.

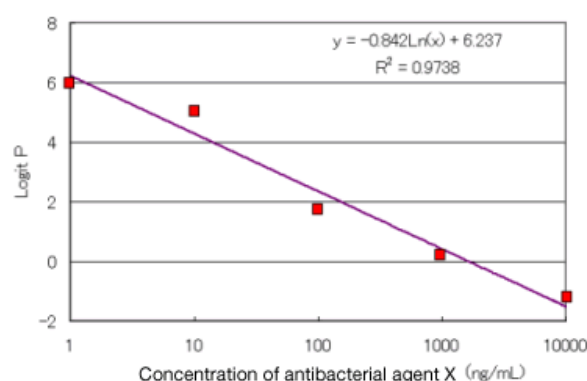
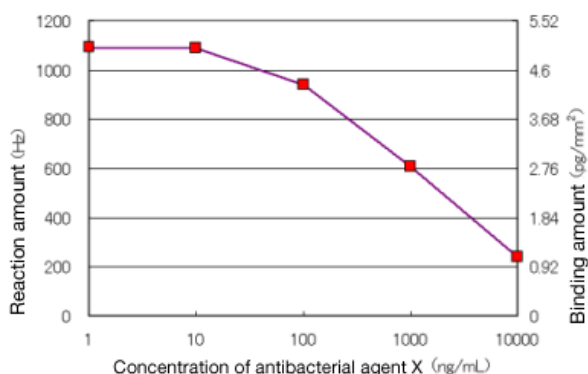


Fig.4: Standard curve of antibacterial agent X

Reaction amount (Hz) & binding amount (pg/mm²) against concentration of antibacterial agent X

Standard curve after Logit transform

● 5. Glossary

- Competitive inhibitory method: Quantification method conducted from signal reduction ration at coexistence between target substance and competitive one.
- Logit transformation: Transform method to shift sigmoid curve (S figure curve) to straight line
- Antibacterial agent: Substance to inhibit bacterial growth or kill bacteria.