

■ Binding transcription factor to DNA

Double-strand DNA (NF-κB target sequence) was immobilized on a sensor, and the reaction of transcription factor (p50) and the signal amplification (sensitization) by antibody were observed.

● 1. Protocol

The measurement was made with the following conditions:

NAPiCOS Auto (Micro fluid flow-cell)

Sensor: 30MHz twin sensor

Flow rate: 2 μL/min

Sample amount: 50 μL

Running buffer: 10mM Tris (pH7.6), 50mM NaCl, 1mM MgCl₂, 0.5mM DTT

Transcriptional factor: NF-κB (p50 homodimer) 5 μg/mL

DNA:

Biotinylated Oligo DNA (25mer)

biotin-5'-CACAGTTGAGGGGACTTTCCAGGC-3'

Complementary DNA (25mer) 10 μg/mL

3'-GTGTCAACTCCCTGAAAGGGTCCG-5'

* The red letters represent NF-κB target sequence.

The above 2 types of synthetic DNA were mixed as total amount 50 μg/mL and double-strand DNA were formed.

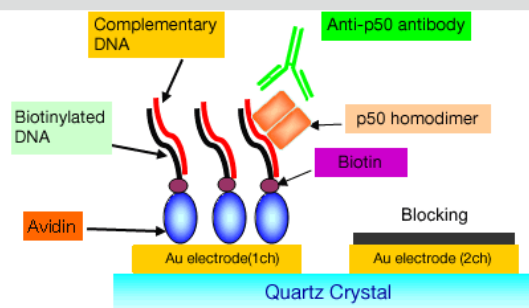


Fig.1: Diagram

Avidin was immobilized on the reaction electrode, and the reference electrode was blocked. After injecting biotinylated double-strand DNA and immobilizing it on the reaction electrode, the binding reaction of p50 was observed. Subsequently, anti p50 antibody was injected and the amplification was observed.

● 2. Reaction waveform

2-1 Biotinylated double-strand DNA was injected and immobilized on the electrode where avidin had been immobilized.

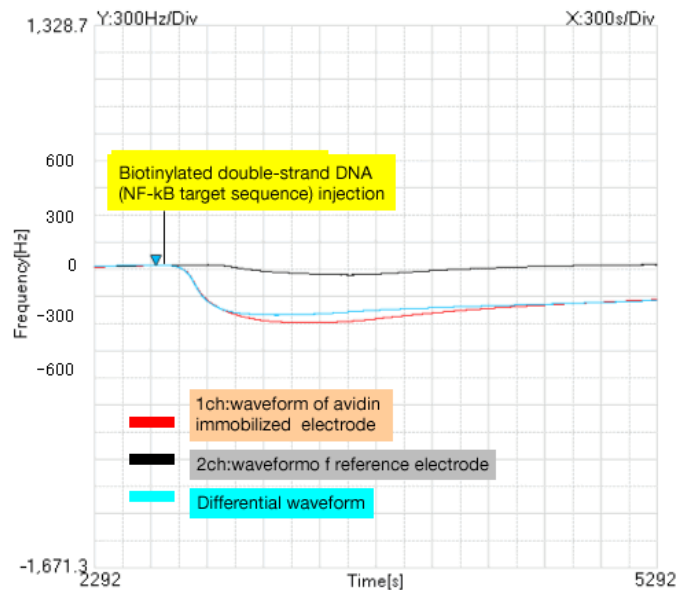


Fig.2: Waveform of biotinylated double-strand DNA

2-2 After the p50 injection, the antibody was injected for amplification.

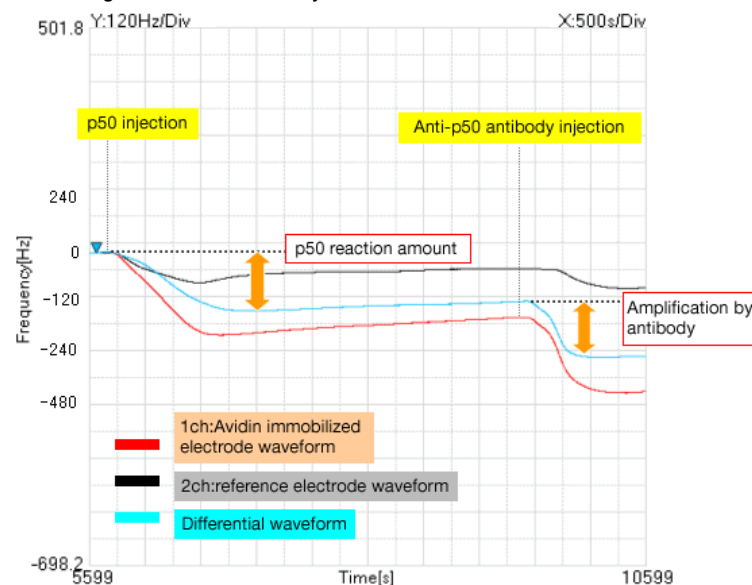


Fig.3: Waveform of p50 and anti p50 antibody reaction

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2-3 For the evaluation of the specificity, biotinylated double-strand DNA with random sequence was immobilized on the avidin electrode and p50 was injected.

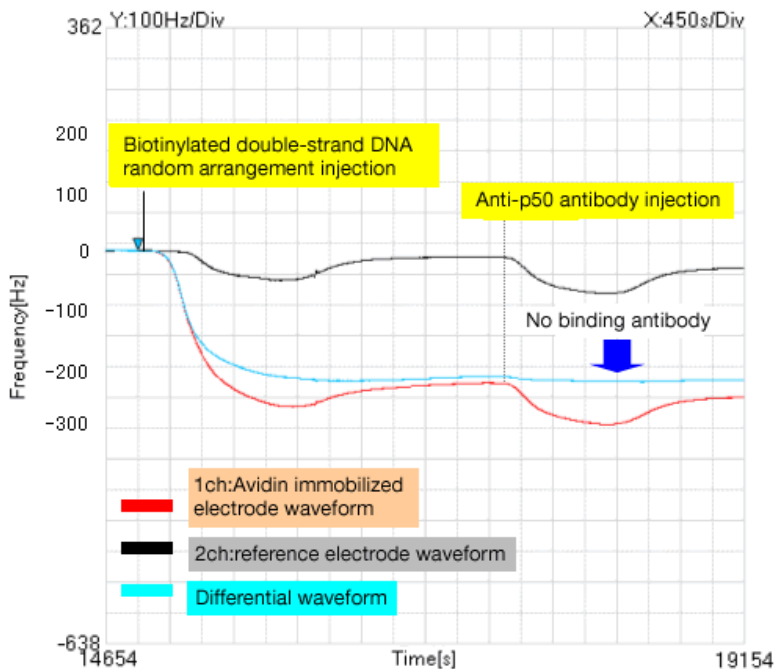


Fig.4:Waveform of immobilized random DNA and p50 injection

● 3.Reaction amount

From the wave form in section2, the difference in the reaction amount was specified. The results showed that p50 specifically bound to NF-kB target sequence.

DNA arrangement	Reaction amount (Hz)	
	Biotinylated double-strand DNA	p50
(1)NF-κB target arrangement	248.57	129.91
(2)Random arrangement	211.15	6.17

Table1: Reaction amount of DNA and p50

*The amount of random double-strand DNA was 20mer and the molecular weight was about 80% of NF-kB double strand DNA. Therefore, the percentage of the reaction amount was also 80.

● 4. Glossary

- Transcriptional factor: The collective term for proteins that bind to DNA. It transcribes the genetic information of DNA to RNA (ribonucleic acid).
- NF-kB (Nuclear Factor Kappa B): NF-kB is a transcriptional factor that consists of p50 (protein with molecular weight of 50,000) and p65 (protein with molecular weight of 65,000) When activated, it binds to the target DNA sequence. p50 binds to p65 to form heterodimer. Also it binds to other p50 to form homodimer.
Since NF-kB prevents the death of cells and accelerates inflammation, it is thought that it increases the spread of cancer
- DTT (Dithiothreitol): Reducing agent with low molecular weight. It protects and stabilizes SH radical.
- Homodimer: A composite consisting of two same substances. A composite consisting of 2 different substances. is called heterodimer.