

formed on the surface of the substrate body composed of silicon dioxide (SiO₂) by the sputtering method. The thin film serves both as the conductive layer and the electron accepting layer. Subsequently, a working electrode lead for connecting to the ammeter was connected to the working electrode body.

[0336] Then, the surface of the working electrode body was brought into contact with the solution A obtained in Preparation example 2-1 to provide a thiol group on the surface of the main body of the working electrode.

[0337] Anti-human interleukin-6 antibody [manufactured by BioLegend] as a trapping substance was reduced by bringing into contact with tris(2-carboxyethyl)phosphine hydrochloride (TCEP) fixed gel as a reducing agent (trade name: Immobilized TCEP Disulfide Reducing Gel, manufactured by Pierce), and a reduced heavy chain of anti-human interleukin-6 antibody was produced. 10 µg/mL of the obtained anti-human interleukin-6 antibody was added to TBS to prepare an antibody solution.

[0338] Then, the obtained antibody solution was dropped onto the working electrode body. The working electrode body was incubated at 4° C. overnight to react the anti-human interleukin-6 antibody with the thiol group on the working electrode body, and a dithiol bond was formed. Thus, the anti-human interleukin-6 antibody was immobilized on the working electrode body. Then, 1 mM triethylene glycol mono-11-mercaptoundecyl ether [manufactured by Sigma] was dropped onto the working electrode body. Blocking was performed by incubating the working electrode body at 4° C. overnight. Thus, a working electrode substrate was obtained.

Example 2-5

[0339] Quantitative Detection of Interleukin-6 (IL-6) with Multivalent-Labeled DNA

(1-1) Trapping of Analyte

[0340] Silicone rubber (0.1 mm in thickness) was placed around the working electrode of the working electrode substrate obtained in Preparation example 2-6 so as to form a partition. Thereafter, TBS-T containing 0.4% by mass of Block Ace [manufactured by DS Pharma Biomedical Co., Ltd.] was poured into the space surrounded by the working electrode substrate and the silicone rubber. The liquid in the above space was discharged. Thereafter, 30 µL of the analyte solution (concentration of the analyte: 500 pg/mL) obtained by diluting human-interleukin-6 as an analyte with TBS-T containing 25% by mass of bovine serum [manufactured by Thermo Scientific] and 0.75% by mass of BSA was added to the above space. Thereafter, the working electrode substrate was incubated at 25° C. for 1 hour to allow the analyte [interleukin-6] to be trapped by the trapping substance [anti-human interleukin-6 antibody].

(1-2) Labeling

[0341] The working electrode substrate subjected to the process (1-1) was washed with TBS-T. Then, biotin-labeled anti-human interleukin-6 antibody [manufactured by BioLegend] was added to TBS-T containing 1% by mass of BSA so that the concentration was 1 µg/mL. Thereafter, 30 µL of the obtained mixture was poured into the above space. Thereafter, the working electrode substrate was incubated at 25° C. for 1 hour. Thus, the biotin-labeled anti-human interleukin-6 antibody (the first conjugate) was bound to the analyte trapped by the trapping substance. The biotin-labeled anti-

human interleukin-6 antibody was obtained by labeling the anti-human interleukin-6 antibody with biotin.

[0342] Then, the working electrode substrate was washed with TBS-T. Then, 2 mg/mL of streptoavidin [manufactured by Vector Laboratories] (the second conjugate) was added to TBS-T so that its concentration was 4 µg/mL. 30 µL of the obtained mixture was poured into the above space. Thereafter, the working electrode substrate was incubated at 25° C. for 30 minutes. Thus, streptoavidin was bound to the biotin-labeled human interleukin-6 antibody on the working electrode.

(1-3) Dye-Labeling

[0343] The working electrode substrate subjected to the process (1-2) was washed with TBS-T. Then, TBS-T was added to 100 µL of a solution containing the biotinylated-DNA/Alexa Fluor 750-labeled DNA complex obtained in Preparation example 2-5 (concentration of the complex: 93 µg/mL) in an amount 10 times the amount of the solution. Thereafter, 30 µL of the obtained mixture was poured into the above space. Thereafter, the working electrode substrate was incubated at 25° C. for 30 minutes. Thus, a complex containing the analyte, the first conjugate, the second conjugate, and the labeled form was formed on the working electrode. The complex formed of the first conjugate and the second conjugate corresponds to the binding substance in the label binding substance obtained in Example 2-1. That is, on the working electrode, Alexa Fluor 750 as the labeling substance is bound to the binding substance bound to the analyte via DNA as the modulator.

(2) Measurement of Photocurrent

[0344] Silicone rubber was placed around the working electrode substrate so that a 0.2-mm-thick side wall was formed. Then, the space surrounded by the working electrode substrate and the silicone rubber was filled with the electrolytic solution obtained in Preparation example 2-3. The space filled with the electrolytic solution was sealed with the counter electrode substrate obtained in Preparation example 2-4 from the upper side of the working electrode substrate. Thus, the working electrode and the counter electrode are brought into contact with the electrolytic solution. Then, the detection chip including the working electrode substrate and the counter electrode was placed in an electrochemical measurement device. The working electrode lead and the counter electrode lead were connected to the ammeter.

[0345] The light source (wavelength: 781 nm, laser light source with an output power of 13 mW) emits excitation light from the working electrode substrate side toward the counter electrode substrate. The labeling substance Alexa Fluor 750 is excited by photoirradiation, thereby generating electrons. When the generated electrons are transported to the working electrode, current flows between the working electrode and the counter electrode. Then, the electric current was measured.

[0346] The current was measured by performing the same operation as described above except that an analyte solution having an analyte concentration of 7.8 pg/mL, 15.6 pg/mL, 31.2 pg/mL, 62.5 pg/mL, 125 pg/mL or 250 pg/mL was used in place of an analyte solution having a concentration of 500 pg/mL in (1-1). The operation was performed in the same