

manner as described above except that the analyte was not used. The control experiment when the analyte was not present was performed.

[0347] FIG. 37 is a graph showing examined results of a relationship between the concentration of the analyte (human IL-6) and photocurrent in Example 2-5.

[0348] From the results shown in FIG. 37, it is found that when the concentrations of the analyte are 7.8 pg/mL, 15.6 pg/mL, 31.2 pg/mL, 62.5 pg/mL, 125 pg/mL, 250 pg/mL, and 500 pg/mL, the photocurrents detected are 0.089 nA, 0.092 nA, 0.095 nA, 0.11 nA, 0.14 nA, 0.21 nA, and 0.3 nA, respectively. It is found that the photocurrent detected when the analyte is not present is 0.083 nA. From these results, it is found that the photocurrent detected by the method for electrochemically detecting an analyte is increased according to the concentration of the analyte and the analyte can be quantitatively detected.

[0349] The above results suggest that analytes other than mouse IgG can be quantitatively detected with high sensitivity by using a multivalent-labeled binding substance in which more labeling substances are immobilized to the binding substance through the interaction between modulators.

Preparation Example 2-7

Production of Working Electrode Substrate

[0350] A working electrode body composed of a thin film (about 200 nm in thickness) of tin-doped indium oxide was 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.

[0351] 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.

[0352] Anti-human interleukin-6 antibody [manufactured by BioLegend] as a trapping substance or anti-human interferon- γ antibody [manufactured by BioLegend] 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 or an anti-human interferon- γ antibody was produced. The obtained anti-human interleukin-6 antibody or anti-human interferon- γ antibody was added to TBS so that the concentration was 10 μ g/mL, and an anti-human interleukin-6 antibody solution or an anti-human interferon- γ antibody solution was obtained.

[0353] Silicone rubber (0.1 mm in thickness) having two openings was placed on the working electrode so as to form a partition. Thereafter, the anti-human interleukin-6 antibody solution was poured into one of the spaces surrounded by the working electrode body and the silicone rubber. The anti-human interferon- γ antibody was placed in the other space. The working electrode body was incubated at 4° C. overnight to react the anti-human interleukin-6 antibody or anti-human interferon- γ antibody with the thiol group on the working electrode body, and a dithiol bond was formed. Thus, the anti-human interleukin-6 antibody and anti-human inter-

feron- γ antibody were immobilized on the working electrode body. The silicone rubber was removed from the working electrode body. Thereafter, silicone rubber (0.1 mm in thickness) having one opening with the size of the range corresponding to the two openings was placed on the working electrode so as to form a partition. Then, 1 mM triethylene glycol mono-11-mercaptopundecyl 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 2-6

[0354] Simultaneous Detection of Interleukin-6 (IL-6) and Interferon- γ with Multivalent-Labeled DNA

(1-1) Trapping of Analyte

[0355] Silicone rubber was placed around the working electrode substrate obtained in Preparation example 2-7 so that a 0.2-mm-thick side wall was formed. Then, 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 μ L of the detecting object solution (concentration of the detecting object: 250 pg/mL) obtained by diluting a single human-interleukin-6 [manufactured by BioLegend] as an analyte, a single human-interferon- γ [manufactured by BioLegend] as an analyte, or a mixture of human-interleukin-6 and human-interferon- γ as analytes with TBS-T containing 25% 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 analytes [interleukin-6 and human-interferon- γ] to be trapped by trapping substances [anti-human interleukin-6 antibody and anti-human interferon- γ antibody].

(1-2) Labeling

[0356] 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] and anti-human interferon- γ antibody [manufactured by BioLegend] were added to TBS-T containing 1% by mass of BSA so that the concentrations thereof were 1 μ g/mL, respectively. 30 μ L 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) and the biotin-labeled anti-human interferon- γ antibody were bound to the analyte trapped by the trapping substance. The biotin-labeled anti-human interleukin-6 antibody and the biotin-labeled anti-human interferon- γ antibody were obtained by respectively labeling the anti-human interleukin-6 antibody and the anti-human interferon- γ antibody with biotin.