

substance so that the concentration was 1 mg/mL, and a solution containing the label binding substance was obtained.

[0281] As for the obtained label binding substance, the number of the labeling substance per molecule of antibody (AlexaFluor750) was examined by measuring the absorption at an absorption wavelength of 749 nm of AlexaFluor750. As a result, it is confirmed that the number of the labeling substance per molecule of antibody (AlexaFluor750) is 9.

Comparative Example 2-1

[0282] Anti-mouse IgG antibody (manufactured by Sigma) was added to 0.1M sodium carbonate buffer (pH 8.5) so that the concentration was 14.6 μ M. Then, a DMSO solution of AlexaFluor750 derivative [trade name: AlexaFluor750 carboxylic acid, succinimidyl ester, manufactured by (Invitrogen)] [concentration of [AlexaFluor750 derivative: 15 mM] was added to the obtained mixture so that the concentration of the AlexaFluor750 derivative was 1.5 mM. The anti-mouse IgG antibody was reacted with the AlexaFluor750 derivative by incubating the obtained mixture at room temperature for 1 hour. The unreacted AlexaFluor750 was removed by subjecting the obtained product to a desalting column [trade name: Zeba Spin Micro desalting Column, manufactured by Pierce] and a labeled antibody was obtained. 0.1M sodium phosphate buffer (pH 7.0) was added to the obtained labeled antibody so that the concentration was 2 mg/mL, and a solution containing the labeled antibody was obtained.

[0283] As for the obtained labeled antibody, the number of the labeling substance per molecule of antibody (AlexaFluor750) was examined by measuring the absorption at an absorption wavelength of 749 nm of AlexaFluor750. As a result, it is confirmed that the number of the labeling substance per molecule of antibody (AlexaFluor750) is 10.

Test Example 2-1

(1-1) Trapping of Analyte

[0284] Silicone rubber (0.1 mm in thickness) was placed around the working electrode of the working electrode substrate obtained in Preparation example 2-2 so that a partition was formed. Thereafter, a tris buffer solution (TBS-T) containing 0.05% by mass of polyoxyethylene sorbitan mono laurate (Tween-20) including 1% by mass of bovine serum albumin (BSA) was poured into the space surrounded by the working electrode substrate and the silicone rubber. Then, after the discharge of the liquid in the space, 30 μ L of TBS-T containing 1% by mass of BSA containing 100 ng/mL mouse IgG (analyte) was added to the above space. Thereafter, the working electrode substrate was incubated at 25° C. for 1 hour to allow the analyte [mouse IgG] to be trapped by the trapping substance [anti-mouse IgG F(ab')₂ antibody] [see the process of trapping an analyte of FIG. 23B].

(1-2) Labeling

[0285] The working electrode substrate was washed with TBS-T. Then, the solution containing 1 mg/mL of the label binding substance obtained in Example 2-1 was added to TBS-T containing 1% by mass of BSA so that the concentration of the label binding substance was 20 μ g/mL. 30 μ L of the obtained mixture was poured into the above space. Thereafter, the analyte on the working electrode was labeled by incubating the working electrode substrate at 25° C. for 1 hour [see FIG. 23C] (Test No. 1).

[0286] On the other hand, the control experiment when no analyte was present was performed as follows. First, the working electrode substrate by which the analyte was not trapped was washed with TBS-T. Then, the solution containing 1 mg/mL of the label binding substance obtained in Example 2-1 was added to TBS-T containing 1% by mass of BSA so that the concentration of the label binding substance was 20 μ g/mL. 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 (Test No. 2).

(2) Control Experiment

[0287] An analyte [mouse IgG] was trapped by a trapping substance [anti-mouse IgG F(ab')₂ antibody] by performing the same operation as (1-1).

[0288] Thereafter, the same operation as (1-2) was performed to label the analyte on the working electrode (Test No. 3) except that the solution containing the labeled antibody obtained in Comparative example 2-1 was used in place of the solution containing the label binding substance obtained in Example 2-1 in (1-2).

[0289] On the other hand, the control experiment when no analyte was present was performed as follows. The working electrode substrate by which the analyte was not trapped was washed with TBS-T. Then, the solution containing 2 mg/mL of the labeled antibody obtained in Comparative example 2-1 was added to TBS-T containing 1% by mass of BSA so that the concentration of the label binding substance was 20 μ g/mL. 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 (Test No. 4).

(3) Measurement of Photocurrent

[0290] Silicone rubber was placed around the working electrode substrates of Test Nos. 1-4 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. Thereafter, 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.

[0291] 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 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 current was measured [see the detection process of FIG. 23D].

[0292] FIG. 27A shows an outline explanatory view showing the detection process when an analyte is detected using the label binding substance obtained in Example 2-1 in Test example 2-1 (Test No. 1). FIG. 27B shows an outline explanatory view showing the detection process when an analyte is detected using the labeled antibody obtained in Comparative