

example 2-1 in Test example 2-1 (Test No. 3). FIG. 28 shows examined results of a relationship between the kind of the detection method and photocurrent in Test example 2-1.

[0293] In the method for electrochemically detecting an analyte of Test No. 1, the label binding substance obtained in Example 2-1 [see 410 in FIG. 27A] is used. Therefore, as shown in FIG. 27A, photocurrents are generated from 9 labeling substances per molecule of antibody by the irradiation of the excitation light in the detection process. On the other hand, in the method for electrochemically detecting an analyte of Test No. 3, the labeled antibody obtained in Comparative example 2-1 [see 421 in FIG. 27B] is used. Thus, as shown in FIG. 27B, photocurrents are generated from 10 labeling substances per molecule of antibody by the irradiation of the excitation light in the detection process. Therefore, it may be predicted that the photocurrent detected by the method for electrochemically detecting an analyte of Test No. 1 is almost the same as or slightly smaller than that detected by the method for electrochemically detecting an analyte of Test No. 3.

[0294] However, from the results shown in FIG. 28, it is found that the photocurrent detected by the method for electrochemically detecting an analyte of Test No. 1 is 20 nA, while the photocurrent detected by the method for electrochemically detecting an analyte of Test No. 3 is 4.5 nA. From these results, it is found that, unexpectedly, the photocurrent detected by the method for electrochemically detecting an analyte of Test No. 1 is far larger than that detected by the method for electrochemically detecting an analyte of Test No. 3.

[0295] As for the label binding substance obtained in Example 2-1 and the labeled antibody obtained in Comparative example 2-1, the same antibody is used as the binding substance. Thus, it is considered that the binding efficiency of the label binding substance obtained in Example 2-1 to the analyte is almost equal to that of the labeled antibody obtained in Comparative example 2-1. In the case where there is no analyte, the photocurrent is about 0 nA (see Test Nos. 2 and 4 in FIG. 28). Thus, it is considered that a difference between the photocurrent detected by the method for electrochemically detecting an analyte of Test No. 1 and the photocurrent detected by the method for electrochemically detecting an analyte of Test No. 3 is not caused by the influence of noise. Therefore, it is considered that a difference between the photocurrent detected by the method for electrochemically detecting an analyte of Test No. 1 and the photocurrent detected by the method for electrochemically detecting an analyte of Test No. 3 is dependent on the presence of DNA between the labeling substance and the binding substance.

Test Example 2-2

Example 2-2

(1-1) Trapping of Analyte

[0296] 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, TBS-T containing 1% by mass of 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 [see FIG. 29A]. 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 FIG. 29B].

(1-2) Labeling

[0297] The working electrode substrate was washed with TBS-T. Then, 4 μ g/mL of a solution containing 2.1 mg/mL biotin-labeled anti-mouse IgG antibody [manufactured by Sigma] was added to TBS-T containing 1% by mass of BSA. 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-mouse IgG antibody (the first conjugate) [see 430a in FIG. 29B] was bound to the analyte [see S in FIG. 29B] trapped by the trapping substance [see 281 in FIG. 29B]. The biotin-labeled anti-mouse IgG antibody is obtained by labeling an anti-mouse IgG antibody [see 431a in FIG. 29B] with biotin [see 431b in FIG. 29B].

[0298] The working electrode substrate was washed with TBS-T. A solution containing streptavidin [manufactured by Vector] as the second conjugate [concentration of the second conjugate: 2 mg/mL] was added to TBS-T so that the concentration of the second conjugate was 4 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 30 minutes. Thus, streptavidin [see 430b in FIG. 29C] was bound to biotin-labeled anti-mouse IgG antibody on the working electrode.

[0299] The working electrode substrate was washed with TBS-T. Then, a solution containing biotinylated AlexaFluor750-labeled DNA [concentration of the labeled form: 100 μ M] as the labeled form was added to TBS-T so that the concentration of the labeled form was 1 μ M. 30 μ L 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 (Test No. 5). The complex formed of the first conjugate and the second conjugate [see 431 in FIG. 29D] corresponds to the binding substance in the label binding substance obtained in Example 2-1. That is, on the working electrode, AlexaFluor750 as the labeling substance [see 433 in FIG. 29D] is bound to the binding substance bound to the analyte [see 431 in FIG. 29D] via DNA as the modulator [see 432 in FIG. 29D].

[0300] The biotinylated AlexaFluor750-labeled DNA is DNA in which the 5' terminal of DNA with a length of 24 nucleotides [5'-AACTACTGTCTTCACGCAGAAAGC-3' (SEQ ID NO: 10), manufactured by Invitrogen] is modified by biotin and the 3' terminal is labeled with AlexaFluor750.

[0301] On the other hand, the operation was performed in the same manner as described above except that the analyte was not used. The control experiment when the analyte was not present in Example 2-2 was performed (Test No. 6).

(2) Control Experiment

Comparative example 2-2

[0302] The labeled antibody to which the labeling substance was directly bound to the antibody [the labeled antibody obtained in Comparative example 2-1] was used in place of the first conjugate, the second conjugate, and the labeled form. The control experiment was performed in the following manner. The same operation as (1-1) was performed to allow