

FIG. 33). The biotinylated-DNA is DNA (DNA whose 5' terminal of phosphate group is a biotinylated adenine base through the  $(\text{CH}_2)_3$  linker) in which the 5' terminal of DNA with a length of 84 nucleotides [5'-biotin-AAACCACGGC-CCTAGGGACAACGACCACGGCCCTAGG-GACAACGACCACGGCCCTAGGG ACAACGACCACG-GCCCTAGGGACAACGA-3', manufactured by Invitrogen, (SEQ ID NO: 11), see 372a in FIG. 33] is labeled with biotin (see 372b in FIG. 33) (see 372 in FIG. 33). The AlexaFluor 750-labeled DNA is DNA in which the 3' and 5' terminals of DNA with a length of 20 nucleotides [5'-CGTTGTC-CCTAGGGCCGTGGGTATGCGCGCTGCTATGCCG-3', manufactured by Invitrogen, (SEQ ID NO: 12), see 371b in FIG. 33] are labeled with AlexaFluor 750 (see 371a in FIG. 33) (see 371 in FIG. 33).

#### Example 2-3

**[0313]** Detection of Mouse IgG with Multivalent-Labeled DNA

##### (1-1) Trapping of Analyte

**[0314]** 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 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. Then, the working electrode substrate was incubated at 25° C. for 30 minutes. After the washing the working electrode substrate with TBS-T, 30  $\mu\text{L}$  of TBS-T containing 1% by mass of BSA containing 10 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 to be trapped by the trapping substance [anti-mouse IgG F(ab')<sub>2</sub> antibody, see 81 in FIG. 34] immobilized on the working electrode body of the working electrode substrate (see 61 in FIG. 34) [see FIG. 34A].

##### (1-2) Labeling

**[0315]** The working electrode substrate subjected to the process (1-1) was washed with TBS-T. Then, biotin-labeled anti-mouse IgG F(ab')<sub>2</sub> antibody [manufactured by Dako] was added to TBS-T containing 1% by mass of BSA so that the concentration of the antibody was 4  $\mu\text{g}/\text{mL}$ . Thereafter, 30  $\mu\text{L}$  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 F(ab')<sub>2</sub> antibody (the first conjugate) (see 351 in FIG. 34) was bound to the analyte (see S in FIG. 34) trapped by the trapping substance (see 81 in FIG. 34) [see FIG. 34B]. The biotin-labeled anti-mouse IgG F(ab')<sub>2</sub> antibody was obtained by labeling anti-mouse IgG F(ab')<sub>2</sub> antibody (see 352 in FIG. 34) with biotin (see 353 in FIG. 34).

**[0316]** Then, the working electrode substrate was washed with TBS-T. Then, streptavidin [manufactured by Vector Laboratories] (the second conjugate) was added to TBS-T so that its concentration was 4  $\mu\text{g}/\text{mL}$ . 30  $\mu\text{L}$  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 360 in FIG. 34) was bound to biotin (353 in FIG. 34) in the biotin-labeled anti-mouse IgG

F(ab')<sub>2</sub> antibody (see 351 in FIG. 34) on the working electrode body (see 61 in FIG. 34) [see FIG. 34C].

##### (1-3) Dye-Labeling

**[0317]** The working electrode substrate subjected to the process (1-2) was washed with TBS-T. Then, TBS-T was added to 30  $\mu\text{L}$  of a solution containing the biotinylated-DNA/Alexa Fluor 750-labeled DNA complex obtained in Preparation example 2-5 (concentration of the complex: 93  $\mu\text{g}/\text{mL}$ ) in an amount 10 times the amount of the solution. Thereafter, 30  $\mu\text{L}$  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 (see S in FIG. 34), the biotin-labeled anti-mouse IgG F(ab')<sub>2</sub> antibody (the first conjugate) (see 351 in FIG. 34), streptavidin (the second conjugate) (see 360 in FIG. 34), and the biotinylated-DNA/Alexa Fluor 750 labeled DNA complex (see 370 in FIG. 34) as a labeled form was formed on the working electrode body (see 61 in FIG. 34) [see FIG. 34D]. 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

**[0318]** 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.

**[0319]** 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 (Test No. 9).

**[0320]** The same operation as above-described was performed by using the complex used in Test No. 5 (biotinylated Alexa Fluor 750-labeled DNA: SEQ ID NO 10) in place of the biotinylated-DNA/Alexa Fluor 750 labeled DNA complex obtained in Preparation example 2-5, and the resulting product was used for the control experiment (Test No. 10).

**[0321]** FIG. 35 shows examined results of a relationship between the kind of the detection method and photocurrent in Example 2-3.

**[0322]** From the results shown in FIG. 35, it is found that the photocurrent detected by the method for electrochemically detecting an analyte of Test No. 9 is 3.6 nA, while the photocurrent detected by the method for electrochemically