

the working electrode was irradiated with ultraviolet rays (160 mJ) to immobilize CK19 DNA-trapping DNA on the working electrode. Thus, a working electrode substrate was obtained.

(1-4) Trapping of Analyte

[0177] Silicone rubber (0.1 mm in thickness) was placed around the working electrode of the working electrode substrate so that a partition was formed. Thereafter, a hybridization buffer [trade name: Perfect Hyb, manufactured by TOYOBO CO., LTD] and 1 nM CK19 DNA [150 bases, SEQ ID NO:5, Manufactured by Hokkaido System Science Co., Ltd.] as the analyte S were placed in the space surrounded by the working electrode substrate and the silicone rubber. The working electrode substrate was incubated at 58° C. for 2 hours. Thus, the analyte S was trapped by the trapping substance [see 81 in FIG. 11A] on the working electrode body [see 61 in FIG. 11A] [see FIG. 11A].

[0178] The working electrode 60 was washed with 6×SSPE containing 0.1% by mass of polyoxyethylene sorbitan mono laurate (Tween-20) [composition of 1×SSPE: 0.01 M phosphate buffer (pH 7.4), 0.149 M sodium chloride, 0.001 M EDTA]. Thereafter, 1×MES hybridization solution (manufactured by Affymetrix) and 50 nM DNA binding ferritin (conjugate) were placed in the above space. The working electrode substrate (the upper substrate 30) was incubated at 45° C. for 1 hour. Thus, the analyte S and the DNA-binding ferritin 110a (conjugate) were trapped on the working electrode body 61 [see FIG. 11B] by hybridizing the analyte S trapped by the trapping substance 81 on the working electrode body 61 with DNA (binding substance) included in the DNA-binding ferritin 110a (conjugate) [see 112 in FIG. 11B]. Here, the analyte S is hybridized with a dashed line portion [the italicized sequence in FIG. 10A] of a DNA 112 (binding substance) included in the DNA-binding ferritin 110a (conjugate).

[0179] Then, the working electrode 60 was washed with 6×SSPE containing 0.1% by mass of Tween-20. Thereafter, 1×MES hybridization solution (manufactured by Affymetrix) and a labeled form 110b were placed in the above space. The working electrode substrate (the upper substrate 30) was incubated at 45° C. for 1 hour. Thus, a complex containing the trapping substance 81, the analyte S, the DNA binding ferritin 110a (conjugate), and the labeled form 110b was formed on the working electrode body 61 of the working electrode substrate (the upper substrate 30) [see FIG. 11C] (Example 1-1).

(2) Control Experiment

[0180] The same operation as Example 1-1 was performed except that 1 μM CK19 recognizing/label-retaining DNA [SEQ ID NO: 6, see FIGS. 10F and 120a in FIG. 12] was used in place of the DNA binding ferritin in Example 1-1. Thus, a complex containing the trapping substance 81, the analyte S, a CK19 recognizing/label-retaining DNA 120a, and the labeled form 110b was formed on the working electrode body 61 of the working electrode substrate (the upper substrate 30) [see FIG. 12C] (Comparative example 1-1).

(3) Measurement of Photocurrent

[0181] Silicone rubber was placed around the working electrode substrates of Example 1-1 and Comparative example 1-1 so that a 0.2-mm-thick side wall was formed. Then, the space surrounded by the working electrode sub-

strate (the upper substrate 30) and the silicone rubber was filled with the electrolytic solution obtained in Preparation example 1-2. The space filled with the electrolytic solution was sealed with the counter electrode substrate obtained in Preparation example 1-3 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.

[0182] The light source (wavelength: 781 nm, laser light source with an output power of 13 mW) emits 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 electric current was measured. FIG. 13 shows examined results of a relationship between the kind of the detection method and photocurrent in Test example 1-1.

[0183] From the results shown in FIG. 13, it is found that the current detected in Example 1-1 in which ferritin (a support composed of polypeptide) is used as the support retaining the labeling substance is about 137 nA. On the other hand, it is found that the current detected in Comparative example 1-1 in which the support composed of polypeptide is not used as the support retaining the labeling substance like a conventional manner is about 17 nA. From these results, it is found that a very large current can be detected when the support composed of polypeptide is used as the support retaining the labeling substance.

Test Example 1-2

(1-1) Production of DNA Binding BSA

[0184] Bovine serum albumin (BSA) (manufactured by Sigma) was purified by gel filtration chromatography. The purified BSA [131 in FIG. 15B] was reacted with a cross-linker [trade name: GMBS, manufactured by Dojin Chemical Laboratory] and a maleimide group was bound to the surface of BSA. 2.6 nmol of the obtained maleimide-modified BSA was mixed with 2.7 nmol of CK19-recognizing DNA having a thiol group at the 3' terminal [SEQ ID NO: 7, 20 bases, see 132 in FIG. 14A and FIG. 15B] and 6.4 nmol of label-retaining DNA-binding DNA having a thiol group at the 3' terminal [SEQ ID NO: 8, see 134 in FIG. 14B and FIG. 15B]. The mixture was reacted in 100 μL of 0.15 M sodium chloride containing phosphoric acid buffer (pH 7) at 37° C. for 2 hours. The obtained reaction product was ultrafiltered through a centrifugal filter [trade name: Amicon Ultra 0.5, 30 K cut-off, manufactured by Millipore] to remove unreacted DNA, and DNA binding BSA [see 130a in FIG. 15B] was obtained.

[0185] The CK19-recognizing DNA has a sequence [the sequence indicated by boldface in FIG. 14A] complementary to CK19 DNA (the analyte S) [SEQ ID NO: 5, FIG. 14F]. The label-retaining DNA-binding DNA has a sequence [the sequence indicated by boldface in FIG. 14B] complementary to a bonding site [the italicized sequence in FIG. 14C] of labeling substance-retaining DNA [SEQ ID NO: 2, see FIG. 14C].

(1-2) Production of Labeled Form

[0186] 100 nM of labeling substance-retaining DNA (the second binding substance) [manufactured by Hokkaido Sys-