

manufactured by Dojin Chemical Laboratory], and a maleimide group was bound to the surface of the AlexaFluor750-labeled BSA.

[0196] The obtained reaction product was purified through a desalting column to remove an unreacted cross-linker. Thereafter, 1 nmol of the maleimide-modified AlexaFluor750-labeled BSA thus obtained was mixed with 2 nmol of CK19-recognizing DNA having a thiol group at the 3' terminal [SEQ ID NO: 7, 20 bases, see FIG. 18A]. The mixture was reacted in 50 μ L of 0.15 M sodium chloride containing phosphoric acid buffer (pH 7) at 37° C. for 3 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 AlexaFluor750-labeled BSA was obtained.

(1-2) Trapping of Analyte

[0197] Silicone rubber (0.1 mm in thickness) was placed around the working electrode **60** of the working electrode substrate (the upper substrate **30**) obtained in a similar manner to (1-3) of Test example 1-1 so that a partition was formed. CK19 DNA-trapping DNA (trapping substance **81**) [see FIG. 18C, SEQ ID NO: 4] was immobilized on the working electrode body **61** of the working electrode substrate (the upper substrate **30**).

[0198] 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., see FIG. 18D] as the analyte S were placed in the space surrounded by the working electrode substrate (the upper substrate **30**) and the silicone rubber. The working electrode substrate (the upper substrate **30**) was incubated at 60° C. for 2 hours. Thus, the analyte S was trapped by the trapping substance **81** on the working electrode body **61** [see of FIG. 19A].

[0199] After discharge of the reaction solution, a hybridization buffer [trade name: Perfect Hyb, manufactured by TOYOBO CO., LTD.] and 100 nM of DNA binding AlexaFluor750-labeled BSA (label binding substance) [see **150** in FIG. 19B] were placed in the above space. The working electrode substrate (the upper substrate **30**) was incubated at 60° C. for 1 hour. Thus, the analyte S trapped by the trapping substance **81** on the working electrode body **61** was hybridized with DNA **152** (binding substance) included in a DNA binding AlexaFluor750-labeled BSA **150** [see of FIG. 19B]. Thereafter, the working electrode **60** was washed with 6xSSPE containing 0.1% by mass of Tween-20 (Example 1-3).

(2) Control Experiment

[0200] The same operation as Example 1-1 was performed except that 100 nM DNA recognizing Alexa Fluor750-labeled CK19 [manufactured by Japan Bio Services Co., LTD., SEQ ID NO: 9, see **160** in FIG. 18B and FIG. 20B] was used in place of the DNA binding AlexaFluor750-labeled BSA **150** in Example 1-3. A complex containing the trapping substance **81**, the analyte S, and the Alexa Fluor750-labeled CK19-recognizing DNA **160** was formed on the working electrode **61** of the working electrode substrate (the upper substrate **30**) [see FIG. 20B] (Comparative example 1-3). The Alexa Fluor750-labeled CK19-recognizing DNA **160** has Alex-

aFluor750 [see **161** FIG. 20B] at both ends of CK19 recognizing DNA [see **162** in FIG. 20B].

(3) Measurement of Photocurrent

[0201] Silicone rubber was placed around the working electrode substrates of Example 1-3 and Comparative example 1-3 so that a 0.2-mm-thick side wall was formed. Then, the space surrounded by the working electrode substrate (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.

[0202] 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. 21 shows examined results of a relationship between the kind of the detection method and photocurrent in Test example 1-3.

[0203] From the results shown in FIG. 21, it is found that the current detected in Example 1-3 in which BSA (a support composed of polypeptide) is used as the support retaining the labeling substance is about 10 nA. On the other hand, it is found that the current detected in Comparative example 1-3 in which the support composed of polypeptide is not used as the support retaining the labeling substance like a conventional manner is about 2.0 nA. From these results, it is found that according to the method for electrochemically detecting an analyte using the support composed of polypeptide as the support retaining the labeling substance, it is possible to detect a current about five times as large as the current detected by the method for electrochemically detecting an analyte not using the support composed of polypeptide.

[0204] As described above, in the DNA binding AlexaFluor750-labeled BSA **150**, the number of AlexaFluor750 added to BSA is about 10. On the other hand, in the Alexa Fluor750-labeled CK19-recognizing DNA, the number of AlexaFluor750 added to DNA is 2. Therefore, the current value to be detected is proportional to the number of labeling. Thus, according to the method for electrochemically detecting an analyte using the support composed of polypeptide, it is suggested that the analyte can be quantified.

Second Embodiment

[Method for Electrochemically Detecting Analyte]

[0205] The method for electrochemically detecting an analyte according to the second embodiment of the present invention is a method for electrochemically detecting an analyte in an electrolytic solution which includes

[0206] (1) bringing a sample containing an analyte into contact with a working electrode on which a trapping sub-