

larly a buffer containing a surfactant); purified water (particularly purified water containing a surfactant); and ethanol can be used.

[0267] In the method for electrochemically detecting an analyte according to the present embodiment, from the viewpoint of removing free label binding substance **390** which is not bound to the analyte **S** and improving the detection accuracy, the process of washing the inside of the detection chip **20** to remove the free label binding substance **390** may be further performed after the labeling process. For example, ethanol and purified water can be used for the washing.

[0268] In the present invention, the operation may be performed so as to form a complex containing the trapping substance **281** on the working electrode body **61**, the analyte **S**, and the label binding substance **390** in the labeling process, in place of labeling the analyte **S** using the label binding substance **390** to which the labeling substance **393** is bound in advance in the labeling process.

[0269] In FIG. 26D, taking the case where the light is measured as an example, the process is illustrated. When the labeling substance **393** is the labeling substance which generates oxidation reduction current when a voltage is applied, the labeling substance **393** is excited to generate electrons. The generated electrons move to the working electrode **60**. As a result, current flows between the working electrode **60** and the counter electrode **66**. Then, the current flowing between the working electrode **60** and the counter electrode **66** is measured by the ammeter **14** of the detector **1**. The current value measured by the ammeter **14** correlates with the number of the labeling substance. Therefore, the analyte can be quantified based on the measured current value.

Second Example

[0270] Hereinafter, the present invention will be described in detail with reference to Examples, however, the present invention is not limited thereto.

Preparation Example 2-1

[0271] 1% by volume of 3-mercaptopropyltriethoxysilane (MPTES), i.e., a silane coupling agent, was added to toluene to prepare a solution A.

Preparation Example 2-2

Production of Working Electrode Substrate

[0272] A working electrode body composed of a thin film (about 200 nm in thickness) of tin-doped indium oxide was formed on the surface of the substrate body composed of silicon dioxide (SiO₂) by the sputtering method. The thin film serves both as the conductive layer and the electron accepting layer. Subsequently, a working electrode lead for connecting to the ammeter was connected to the working electrode body.

[0273] Then, the surface of the working electrode body was brought into contact with the solution A obtained in Preparation example 2-1 to provide a thiol group on the surface of the main body of the working electrode.

[0274] Anti-mouse IgG F(ab')₂ antibody as a trapping substance (manufactured by Dako) was reduced by bringing into contact with tris(2-carboxyethyl)phosphine hydrochloride (TCEP) fixed gel as a reducing agent (trade name: Immobilized TCEP Disulfide Reducing Gel, manufactured by Pierce), and an anti-mouse IgG Fab antibody was produced. 10 μg/mL of the obtained anti-mouse IgG Fab antibody was

added to a tris buffer solution [pH 7.2, hereinafter called "TBS"] to prepare an antibody solution.

[0275] Then, the obtained antibody solution was dropped onto the working electrode body. The working electrode body was incubated at 4° C. overnight to react the anti-mouse IgG Fab antibody with the thiol group on the working electrode body, and a dithiol bond was formed. Thus, the anti-mouse IgG Fab antibody was immobilized on the working electrode body. Then, 1 mM triethylene glycol mono-11-mercaptopoundecyl ether [manufactured by Sigma] was dropped onto the working electrode body. Blocking was performed by incubating the working electrode body at 4° C. overnight. Thus, a working electrode substrate was obtained.

Preparation Example 2-3

[0276] Acetonitrile and ethylene carbonate were mixed at a volume ratio of 2:3 to prepare an aprotic solvent. As an electrolyte salt, tetrapropylammonium iodide was dissolved in the aprotic solvent at a concentration of 0.6 M. As an electrolyte, iodine was dissolved in the obtained solution at a concentration of 0.06 M to prepare an electrolytic solution.

Preparation Example 2-4

[0277] A counter electrode of a 200-nm thick platinum thin film (conductive layer) was formed on the substrate body of silicon dioxide (SiO₂) by the sputtering method to obtain a counter electrode substrate. The counter electrode lead for connecting to the ammeter was connected to the counter electrode. Thus, the counter electrode substrate was obtained.

Example 2-1

[0278] Anti-mouse IgG antibody (manufactured by Sigma) was added to 0.1M sodium phosphate buffer (pH 7) so that the concentration was 7.3 μM. Then, a dimethyl sulfoxide (DMSO) solution of a cross-linker [N-(4-maleimidebutyryloxy)succinimide (GMBS), manufactured by Dojin Chemical Laboratory] [concentration of the cross-linker: 25 mM] was added to the obtained mixture so that the concentration of the cross-linker was 2.5 mM. The anti-mouse IgG antibody was reacted with GMBS by incubating the obtained mixture at room temperature for 30 minutes. The unreacted GMBS was removed by subjecting the obtained product to a desalting column [trade name: Zeba Spin Micro desalting Column, manufactured by Pierce] and a cross-linker-binding antibody was obtained.

[0279] The cross-linker-binding antibody thus obtained was mixed with AlexaFluor750-labeled thiolated DNA so that a molar ratio (a succinimide group introduced antibody/AlexaFluor750-labeled thiolated DNA) was 1/14. The cross-linker-binding antibody was reacted with the AlexaFluor750-labeled thiolated DNA by incubating the obtained mixture at room temperature for 4 hours. The AlexaFluor750-labeled thiolated DNA is DNA in which the 5' terminal of DNA with a length of 24 nucleotides [5'-AACTACTGTCTTCACGCA-GAAAGC-3' (SEQ ID NO: 10), manufactured by Invitrogen] is labeled with AlexaFluor750 and the 3' terminal is modified by a thiol group.

[0280] The unreacted AlexaFluor750-labeled thiolated DNA was completely removed by filtering the obtained product through an ultrafiltration column [trade name: Amicon Ultra-0.5 100K, manufactured by Amicon]-4 times, and a label binding substance was obtained. 0.1M sodium phosphate buffer (pH 7.0) was added to the obtained label binding