

ANTIBODY COMPOSITION AND METHOD FOR PRODUCING THE SAME

BACKGROUND OF THE INVENTION

5 Fields of the invention

【0001】 The present invention relates to an antibody composition and a method for producing the same, especially to an antibody composition containing at least
10 one monoclonal antibody and a method for producing the same by which the monoclonal antibody against type 1 to type 4 dengue fever is produced by hybridoma cell techniques and is used to detect type 1 to type 4 dengue fever.

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Descriptions of Related Art

【0002】 With the increase in global transport and communications, vector-borne infectious diseases are spread
20 rapidly around the world due to worldwide movement of pathogens and carriers. A group of viruses that are transmitted by anthropod vectors such as mosquitoes and ticks and called arthropod-borne virus (Arbovirus) causes serious infection. There are Among over 700 known
25 arboviruses and about 100 arboviruses that cause infections in humans. Among these viruses, flaviviruses and alphaviruses are both of the most important public health issue. Several major human pathogenic flaviviruses containing dengue fever virus, yellow fever virus, Japanese
30 encephalitis virus, West Nile fever/encephalitis virus, St. Louis encephalitis virus, tick-borne encephalitis virus (TBEV), etc.. Alphaviruses cause diseases ranging from chikungunya, Ross River fever, Eastern Equine encephalitis

(EEE), Western Equine encephalitis (WEE), Venezuelan equine encephalitis (VEE), etc.

5 **【0003】** Dengue virus is transmitted to human mainly by *Aedes aegypti* and *Aedes albopictus* mosquitoes. The *Aedes aegypti* appears only south of the Tropic of Cancer, while the *Aedes albopictus* is distributed throughout Taiwan.

10 **【0004】** Dengue fever is the most important vector-borne infectious diseases in Taiwan caused by arthropod vectors and is not considered endemic in Taiwan due to the constant importation of DENVs from the neighboring Southeast Asian countries through close commercial links while air travel is responsible for the local outbreaks each year. Dengue fever
15 is the most common arboviral disease in tropical and sub-tropical regions of the world, with more than 50 million cases recorded every year and leading to half a million hospitalizations and approximately 25,000 deaths. Dengue is endemic in South-East Asia countries including Vietnam,
20 Indonesia, Thailand, Philippines and so on.

【0005】 In recent decades, the number of countries with endemic Dengue and the cases reported worldwide have increased dramatically. To prevent and control of dengue
25 fever, humanity still faces many challenges, there is no specific treatment, there is no safe and effective vaccine, but to take timely measures could significantly reduce the clinical treatment of dengue hemorrhagic fever morbidity and mortality, and therefore the treatment of dengue fever
30 largely depends on early diagnosis of infection. But most of the dengue virus infection early lack of specific clinical manifestations, just fever, chills and other flu-like symptoms, fever and other difficult diseases and hemorrhagic fevers distinction must rely on diagnostic

laboratories to confirm. Currently dengue laboratory diagnostic methods include virus isolation, antibody detection and nucleic acid detection. Dengue virus infection virus isolation laboratory diagnosis and serotype
5 identification of the gold standard, but the method is time-consuming and demanding for the laboratory conditions. Although the viral nucleic acid detection method is more sensitive than conventional rapid virus isolation method, but the operation is relatively complicated
10 molecular diagnostics, high level of technical requirements.

【0006】 The development of a rapid and sensitive test kit for early detection of dengue virus to prevent and control of dengue fever is quite important. Denguevirus nonstructural
15 protein 1 (nonstructural protein 1, NS1) is a relatively conservative glycoprotein, molecular weight 45~50kDa, have membrane and secretory two forms, is highly expressed in infected cells, have type-specific determinants, therefore several Studies indicate that the detection of patients with
20 acute serum circulating NSI antigens may be used for early diagnosis of dengue virus infection. The present invention screens highly specific monoclonal antibody for development of Dengue NS1 antigen assay and produces the monoclonal antibody by hybridoma techniques. At least one
25 antibody revealed in the present invention is used to develop the test kit for rapid and sensitive detection of dengue fever and screening of suspected people returned from high-risk area and early detection of infection. At the same time, the serotype can also be confirmed for proper medical care,
30 early reporting, control and prevention of the infection. The kit has great help in control and prevention of dengue fever. The concentration of NS1 protein in the serum of infected patients is ranging from 1 ng/ml to 20 µg/ml. Thus NS1 antigen is an ideal target for detection of dengue virus.

SUMMARY

5 **【0007】** Therefore it is an object of the present invention to provide monoclonal antibody that binds specifically to dengue virus NS1 antigen. It can be effectively employed to detect dengue virus infection in a subject, such as a human. Such assays can be employed to detect the presence of dengue virus infection using biological samples including
10 blood, serum, plasma, saliva, cerebrospinal fluid.(Monique da Rocha Queiroz Lima et al., PLOSNTDS, v5 (5), e1147, 2011).In one example, the disclosed assays employ a first antibody specific for dengue NS1 as a capture antibody, with a second antibody specific for dengue NS1 in monomeric and/or
15 oligomeric (primarily dimeric) forms being employed as a detection antibody.

20 **【0008】** Another object of the present invention is to provide an antibody composition having at least one monoclonal antibody that binds specifically to dengue antigen for detection of dengue NS1 antigen. Thus a monoclonal antibody of the antibody composition can be mass-produced and applied to detect dengue pathogens.

25 **【0009】** In order to achieve the above objects, an antibody composition of the present invention includes at least one monoclonal antibody. In one aspect, there is provided an antibody composition comprising a first monoclonal antibody, a second monoclonal antibody, a third monoclonal
30 antibody, and a fourth monoclonal antibody; wherein the first monoclonal antibody includes heavy chain complementarity determining region 1(HCDR1) of sequence ID number (SEQ ID NO):26, heavy chain complementarity determining region 2(HCDR2) of SEQ ID NO:28, heavy
35 chain complementarity determining region 3(HCDR3) of SEQ ID NO:30, light chain complementarity determining region 1(LCDR1) of SEQ ID NO:32, light chain complementarity determining region 2(LCDR2) of SEQ ID NO:34, light chain complementarity determining region
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3(LCDR3) of SEQ ID NO:36; the second monoclonal antibody includes HCDR1 of SEQ ID NO:62, HCDR2 of SEQ ID NO:64, HCDR3 of SEQ ID NO:66, LCDR1 of SEQ ID NO:68, LCDR2 of SEQ ID NO:70, LCDR3 of SEQ ID NO:72; the third monoclonal antibody includes HCDR1 of SEQ ID NO:74, HCDR2 of SEQ ID NO:76, HCDR3 of SEQ ID NO:78, LCDR1 of SEQ ID NO:80, LCDR2 of SEQ ID NO:82, LCDR3 of SEQ ID NO:84; the fourth monoclonal antibody includes HCDR1 of SEQ ID NO:50, HCDR2 of SEQ ID NO:52, HCDR3 of SEQ ID NO:54, LCDR1 of SEQ ID NO:56, LCDR2 of SEQ ID NO:58, LCDR3 of SEQ ID NO:60; . The monoclonal antibody specifically binds to a

At last produce a monoclonal antibody by the hybridoma cell. The dengue fever can be type 1 dengue fever, type 2 dengue fever, type 3 dengue fever, or type 4 dengue fever. The monoclonal antibody produced can be used to detect the dengue fever.

In another aspect, there is also provided a detection method for detecting dengue virus including the steps of: coating at least one capture monoclonal antibody at the bottom of plurality of sample wells, wherein the at least one capture monoclonal antibody comprises an antibody which includes HCDR1 of SEQ ID NO:38, HCDR2 of SEQ ID NO:40, HCDR3 of SEQ ID NO:42, LCDR1 of SEQ ID NO:44, LCDR2 of SEQ ID NO:46, LCDR3 of SEQ ID NO:48;

adding a sample, a positive control or a negative control in the sample wells respectively and then a dengue antigen in the samples and the positive control bind to the capture monoclonal antibody at the bottom of the sample wells;

adding at least one labeled monoclonal antibody in the sample wells respectively and the labeled monoclonal antibody bind to the dengue antigens captured by the capture monoclonal antibodies, wherein the labeled monoclonal antibody are an antibody which includes HCDR1 of SEQ ID NO:50, HCDR2 of SEQ ID NO:52, HCDR3 of SEQ ID NO:54, LCDR1 of SEQ ID NO:56, LCDR2 of SEQ ID NO:58, LCDR3 of SEQ ID NO:60, an antibody which includes HCDR1 of SEQ ID NO:26, HCDR2 of SEQ ID NO:28, HCDR3 of SEQ ID NO:30, LCDR1 of SEQ ID NO:32,

LCDR2 of SEQ ID NO:34, LCDR3 of SEQ ID NO:36, an antibody which includes HCDR1 of SEQ ID NO:62, HCDR2 of SEQ ID NO:64, HCDR3 of SEQ ID NO:66, LCDR1 of SEQ ID NO:68, LCDR2 of SEQ ID NO:70, LCDR3 of SEQ ID NO:72 or an antibody which includes HCDR1 of SEQ ID NO:74, HCDR2 of SEQ ID NO:76, HCDR3 of SEQ ID NO:78, LCDR1 of SEQ ID NO:80, LCDR2 of SEQ ID NO:82, LCDR3 of SEQ ID NO:84; and

detecting the absorbance of the samples;

wherein the detection method is used to detect type 1 dengue antigens, type 2 dengue antigens, type 3 dengue antigens or type 4 dengue antigens.

In another aspect, there is provided a kit comprising a first monoclonal antibody, a second monoclonal antibody, a third monoclonal antibody, a fourth monoclonal antibody, a fifth monoclonal antibody, and a sixth monoclonal antibody, when used according to the method of claim 2 or 3, wherein the first monoclonal antibody includes HCDR1 of sequence ID number (SEQ ID NO):26, HCDR2 of SEQ ID NO:28, HCDR3 of SEQ ID NO:30, LCDR1 of SEQ ID NO:32, LCDR2 of SEQ ID NO:34, LCDR3 of SEQ ID NO:36; the second monoclonal antibody includes HCDR1 of SEQ ID NO:62, HCDR2 of SEQ ID NO:64, HCDR3 of SEQ ID NO:66, LCDR1 of SEQ ID NO:68, LCDR2 of SEQ ID NO:70, LCDR3 of SEQ ID NO:72; the third monoclonal antibody includes HCDR1 of SEQ ID NO:74, HCDR2 of SEQ ID NO:76, HCDR3 of SEQ ID NO:78, LCDR1 of SEQ ID NO:80, LCDR2 of SEQ ID NO:82, LCDR3 of SEQ ID NO:84; the fourth monoclonal antibody includes HCDR1 of SEQ ID NO:50, HCDR2 of SEQ ID NO:52, HCDR3 of SEQ ID NO:54, LCDR1 of SEQ ID NO:56, LCDR2 of SEQ ID NO:58, LCDR3 of SEQ ID NO:60; the fifth monoclonal antibody includes HCDR1 of SEQ ID NO:2, HCDR2 of SEQ ID NO:4, HCDR3 of SEQ ID NO:6, LCDR1 of SEQ ID NO:8, LCDR2

of SEQ ID NO:10, LCDR3 of SEQ ID NO:12; and the sixth monoclonal antibody specifically includes HCDR1 of SEQ ID NO:38, HCDR2 of SEQ ID NO:40, HCDR3 of SEQ ID NO:42, LCDR1 of SEQ ID NO:44, LCDR2 of SEQ ID NO:46,
5 LCDR3 of SEQ ID NO:48.

In a further aspect, there is provided a kit comprising a first monoclonal antibody, a second monoclonal antibody, a third monoclonal antibody and a fourth monoclonal antibody, when used according to the method of claim 2 or 3, wherein
10 the first monoclonal antibody includes HCDR1 of sequence ID number (SEQ ID NO):14, HCDR2 of SEQ ID NO:16, HCDR3 of SEQ ID NO:18, LCDR1 of SEQ ID NO:20, LCDR2 of SEQ ID NO:22, LCDR3 of SEQ ID NO:24; the second monoclonal antibody includes HCDR1 of SEQ ID
15 NO:50, HCDR2 of SEQ ID NO:52, HCDR3 of SEQ ID NO:54, LCDR1 of SEQ ID NO:56, LCDR2 of SEQ ID NO:58, LCDR3 of SEQ ID NO:60; the third monoclonal antibody includes HCDR1 of SEQ ID NO:2, HCDR2 of SEQ ID NO:4, HCDR3 of SEQ ID NO:6, LCDR1 of SEQ ID NO:8, LCDR2
20 of SEQ ID NO:10, LCDR3 of SEQ ID NO:12; and the fourth monoclonal antibody specifically includes HCDR1 of SEQ ID NO:38, HCDR2 of SEQ ID NO:40, HCDR3 of SEQ ID NO:42, LCDR1 of SEQ ID NO:44, LCDR2 of SEQ ID NO:46, LCDR3 of SEQ ID NO:48.

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【0011】 The type 1 dengue fever, type 2 dengue fever, type 3 dengue fever, and type 4 dengue fever mentioned above represents each of four different serotypes of the dengue virus-being infected by dengue virus type 1, dengue virus
30 type 2, dengue virus type 3, and dengue virus type 4 respectively, and each of which is able to be detected by at least one monoclonal antibody.

【0012】 The dengue antigen can be selected from the
35 following: a virus particle of dengue virus type 1, a virus particle of dengue virus type 2, a virus particle of dengue virus type 3, a virus particle of dengue virus type 4, a dengue virus type 1 NS1 antigen, a dengue virus type 2 NS1

antigen, a dengue virus type 3 NS1 antigen, a dengue virus
type 4 NS1 antigen, recombinant dengue virus type 1 NS1
protein, recombinant dengue virus type 2 NS1 protein,
recombinant dengue virus type 3 NS1 protein, and
5 recombinant dengue virus type 4 NS1 protein.

BRIEF DESCRIPTION OF THE DRAWINGS

10 【0013】 The structure and the technical means adopted by
the present invention to achieve the above and other objects
can be best understood by referring to the following detailed
description of the preferred embodiments and the
accompanying drawings, wherein:

15 Fig. 1 is a flow chart showing steps of an embodiment
according to the present invention;

Fig. 2 shows test results of a dengue fever enzyme-linked
immunoassay kit produced according to the present
invention;

20 Fig. 3 shows an immunochromatography kit produced by an

embodiment according to the present invention;
Fig. 4A shows protein sequence of heavy chain fragment
antigen binding region; and
Fig. 4B shows protein sequence of light chain fragment
5 antigen binding region.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

10 **【0014】** In order to learn features and functions of the present invention, please refer to following embodiments.

【0015】 An antibody composition and a method for producing the same of the present invention features on that
15 the monoclonal antibody contained in the antibody composition can be used to detect different types of dengue fever- type 1 dengue fever, type 2 dengue fever, type 3 dengue fever, and type 4 dengue fever. The monoclonal antibody is produced by using dengue antigen of dengue
20 fever to immunize an experimental animal. The dengue antigen includes virus particle, a dengue virus nonstructural protein 1 antigen (NS1 antigen), and a recombinant dengue virus NS1 protein. Then isolate splenocytes from the experimental animal producing high titer monoclonal
25 antibody and fuse a splenocyte with a tumor cell to form a hybridoma cell. Thus the monoclonal antibody contained in the antibody composition of the present invention is mass-produced by the hybridoma cell and applied to detection of dengue fever.

30 **【0016】** In an embodiment of the antibody composition, the antibody composition includes at least one monoclonal antibody that specifically binds to a dengue antigen of a type of dengue fever. The dengue fever can be type 1 dengue
35 fever, type 2 dengue fever, type 3 dengue fever, or type 4 dengue fever, respectively represents each of four different serotypes of the dengue virus-being infected by dengue virus type 1, dengue virus type 2, dengue virus type 3, and dengue

virus type 4. The present invention can be applied to detect one of the four serotypes of the dengue virus or all the four serotypes of the dengue virus at the same time.

5 **【0017】** Refer to Fig. 1, a method for producing monoclonal antibody of the present invention includes the following steps.

10 **【0018】** Step S10: Provide a dengue antigen of dengue fever.

【0019】 Step S20: Immunize an experimental animal with the dengue antigen.

15 **【0020】** Step S30: Form a hybridoma cell by fusing a splenocyte from the experimental animal with a tumor cell.

【0021】 Step S40: Produce a monoclonal antibody by the hybridoma cell.

20 **【0022】** In the step S10, the dengue antigen provided can be a virus particle of dengue fever, a dengue virus nonstructural protein 1 antigen (NS1 antigen), or a recombinant dengue NS1 protein antigen. The dengue virus NS1 antigen is obtained by purification of dengue virus cell
25 culture medium. Escherichia coli, (E. coli) or other expression systems are used for production of recombinant dengue NS1 antigen.

30 **【0023】** The dengue virus NS1 antigen is prepared by the following method. A Vero cell line (African Green Monkey Kidney Cells) is infected by a dengue virus strain and cell culture medium is collected. Use a centrifugal filter tube to concentrate the cell culture medium containing the dengue
35 virus NS1 antigen and yield a concentrated solution of dengue virus. After virus particles of the dengue virus strain in the concentrated solution of dengue virus being precipitated, a concentrated solution of NS1 antigen is

obtained. A NS1 antibody immunoaffinity chromatography (IAC) column is used for purification of the concentrated solution of NS1 antigen to yield the dengue virus NS1 antigen.

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【0024】 The centrifugal filter tube is a Centricon Plus-10 Centrifugal Filter Device produced by Millipore Company. The cell culture medium is put into the centrifugal filter tube and centrifuged at 3,500 rpm for 10 min to remove protein passed through a filter of the centrifugal filter tube and having a molecular weight smaller than 10 kDa. Then the filter is reversed, connected to a retentate cup and centrifuged at the speed smaller than 2,000 rpm for 10 min. Thus the concentrated solution of dengue virus unable to pass the filter is collected in the retentate cup.

【0025】 A polyethylene glycol (PEG) virus precipitation kit is used to precipitate the virus particles of the dengue virus strain contained in the concentrated solution of dengue virus and is able to be BioVision's PEG virus precipitation kit. After adding the reagents of the PEG virus precipitation kit into the concentrated solution of dengue virus and reacted overnight, the solution is centrifuged at 3,800 rpm for 30 min at 4°C to precipitate the virus particles of the dengue virus strain. The supernatant after precipitation is the concentrated solution of NS1 antigen.

【0026】 In order to produce the NS1 antibody IAC column for purification of NS1 antigen, a protein A affinity chromatography column such as Bio-Rad's Affi-prep protein A cartridges is used to capture a specific anti-NS1 antibody (D0001). After yielding the anti-NS1 antibody, a sugar molecule of the specific anti-NS1 antibody is oxidized and then connected to Bio-Rad's Affi-Gel Hz gel to prepare the NS1 antibody IAC column for purification of NS1 antigen.

【0027】 Before using the NS1 antibody IAC column for purification of dengue virus NS1 antigen contained in the

concentrated solution of NS1 antigen, the concentrated solution of NS1 antigen is added with 0.05% surfactant Tween-20 and aprotinin/or cocktail protease inhibitor. Then the concentrated solution of NS1 antigen is passed through the IAC column, allowing dengue virus NS1 antigen contained in the concentrated solution of NS1 antigen to bind to the specific anti-NS1 antibody in the IAC column. Next a wash buffer is added to the IAC column to remove impurities and an elution buffer is used to elute the dengue virus NS1 antigen from the IAC column for getting the purified dengue virus NS1 antigen. Use spectrometer or protein assay to quantify the dengue virus NS1 antigen, determine the molecular weight by using 4-12% SDS-PAGE electrophoresis, and then store the dengue virus NS1 antigen at -80°C.

【0028】 The recombinant dengue NS1 protein antigen is prepared by the following method: yield at least one DNA fragment of dengue virus NS1 protein of a dengue virus strain and clone it into a pET expression system in E coli to produce a recombinant NS1 protein whose N-terminal (or C-terminal) is added with a hexa histidine-tag (His-tag). Screen an E. coli colony that expresses the recombinant protein with specific epitopes, grow in mass culture and induce the bacteria in the E. coli colony to express the recombinant protein. Use a His-tag affinity chromatography column for purification of the recombinant protein from crude protein extract of the bacteria that are induced to express the recombinant protein.

【0029】 The dengue virus strain is selected from local strain of D1-8700828, D2-454009, D3-8700829 or D4-9201818 respectively belonged to each of four different serotypes of the dengue virus. Add a primer into the whole

or a part of the dengue virus NS1 DNA fragment of the dengue virus strain to perform polymerase chain reaction (PCR) or real-time PCR (RT-PCR) for amplification. Then the 5' end and 3' end of the whole or a part of the sequence
5 of the dengue virus NS1 DNA is added with Bam HI and Sal I cutting sites of restriction enzyme. This DNA fragment is cloned to an expression vector-pET-47b by the restriction enzymes-Bam HI and Sal I so as to produce the recombinant dengue NS1 protein with the His-tag at the N-terminal (or
10 C-terminal). The pET expression system is selected from the Novagen pET system for expression of the recombinant NS1 protein with the His-tag in *E. coli*. Next select a plurality of *E. coli* colonies being cloned. Use a His-tag antibody (as a positive control), an anti-NS1 antibody, one another
15 antibody (as a negative control), etc to perform various tests such as Western blot, immunoprecipitation, etc. to confirm whether the recombinant protein expressed by respective *E. coli* colony carries the specific epitope. At least one *E. coli* colony that expresses the recombinant protein with the
20 specific epitope is obtained after screening. Culture the *E. coli* colony in liquid medium to yield a culture medium of *E. coli* and use a final concentration of 80M isopropyl- β -D-1-thiogalactopyranoside (IPTG) to induce the bacteria in the culture medium of *E. coli* for expression of
25 the recombinant protein.

【0030】 The culture medium of *E. coli* is centrifuged at 14,000 rpm for 10 min. Then add lysis buffer including 10mM Tris-HCl, 8M urea, 100mM NaH₂PO₄, and 5mM
30 imidazol into the solution and adjust the pH value to 8.0 for lysis of bacteria in the culture medium of *E. coli*. . After reacting at 4°C for an hour, the solution is centrifuged at 14,000 rpm for 10 min and a supernatant is collected. Then use the His-tag affinity chromatography column to purify the

recombinant protein in the supernatant.

【0031】 The His-tag affinity chromatography column can be produced by Novagen's Ni-NTA His•Bind® Resin. The supernatant is passed through the His-tag affinity chromatography column twice so that the recombinant protein in the supernatant binds to the Ni-NTA His•Bind® Resin through the His-tag. Then a wash buffer (pH 8.0) including 10mM Tris-HCl, 8M urea, and 100mM NaH₂PO₄ is used to wash the His-tag affinity chromatography column for removing impurities. While washing, add 10mM imidazole and 10 mL wash buffer to wash the His-tag affinity chromatography column twice. Then add 20mM imidazole and 8 mL wash buffer, 40mM imidazole and 6 mL wash buffer respectively for washing the His-tag affinity chromatography column. At last, use an elution solution including 10mM Tris-HCl, 8M urea, 100mM NaH₂PO₄, and 500mM imidazole to elute the recombinant protein from the His-tag affinity chromatography column. 10 mL elution solution is used to elute the recombinant protein with the His-tag at the N-terminal (or C-terminal). After being purified by the His-tag affinity chromatography column, the recombinant protein is dialyzed in a refolding buffer having 50 mM Tris, 400 mM L-arginine, 1.0mM glutathione (GSH), and 0.1 mM glutathione disulfide (GSSG) at 4°C for 3 days. The refolding buffer is replaced at least twice within the 3-day period. At last, use pH7.4 phosphate buffer to dialysis for 8 hours and the recombinant protein treated by dialysis is stored at -20°C. Use Bradford method to quantify the recombinant protein and measure the concentration of the recombinant protein at O.D 595 nm.

【0032】 In the step S20, use the dengue antigen to immunize an experimental animal. As mentioned above, the

dengue antigen can be a virus particle of dengue fever, a dengue virus NS1 protein, or a recombinant dengue NS1 protein. A BALB/c female mouse is used as the experiment animal.

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【0033】 The BALB/c female mouse is immunized by the dengue antigen in the following way. Deliver the dengue antigen into the BALB/c female mouse by intra-peritoneal or subcutaneous injection to stimulate immune responses of the
10 BALB/c female mouse. The injection amount of the dengue antigen at the first time is 50 μg or 10^6 pfu with an equivalent volume of adjuvant which is Sigma's Freund's complete adjuvant. The second and the third injections are added three weeks and six weeks respectively after the first
15 injection. The amount of the second injection and the third injection is still 50 μg or 10^6 pfu while the equivalent volume of adjuvant is replaced by an equivalent volume of Freund's incomplete adjuvant produced by Sigma. The fourth injection is given by intravenous injection, 9 weeks
20 after the first injection. After 50 μg or 10^6 pfu dengue antigen without adjuvant being injected into BALB/c female mice, take a blood sample from the BALB/c female mouse and detect titer of the dengue fever antibody in the serum of the blood sample by Enzyme-linked immunosorbent assay
25 (ELISA).

【0034】 In the step S30, a splenocyte is taken from the experimental animal and fused with a tumor cell to generate a hybridoma cell that secretes a monoclonal antibody against
30 the dengue antigen. The tumor cell can be a FO mouse myeloma cell.

【0035】 In practice, the hybridoma cell that secretes a monoclonal antibody against the dengue antigen is prepared

by the following method. A plurality of splenocytes is removed from the BALB/c female mouse by aseptic technique. The BALB/c female mouse produces high titer antibody against the dengue antigen after being immunized
5 by the dengue antigen. The splenocytes and the FO myeloma cells are induced and fused by polyethylene glycol to generate a plurality of hybridoma cells. The splenocytes include a plurality of B cells able to produce antibody against the dengue antigen. The hybridoma cells prepared by
10 cell fusion of the B cells and the FO myeloma cells have the capacity to produce antibody against the dengue antigen contributed by B cells and the ability to form secondary cultures like tumor cells.

15 **【0036】** The FO myeloma cells are proliferated and cultured 4-7 days before the cell fusion. Thus the FO myeloma cells are at the log phase at the day of cell fusion and the number of the FO myeloma cells is more than 3×10^7 . The splenocytes are washed 3 times by incomplete RPMI
20 culture medium after being removed from the mouse. Then the splenocytes are mixed with the FO myeloma cells and centrifuged carefully to collect the cells at the bottom of a centrifuge tube. Remove the supernatant, use a little amount of PRMI to mix the splenocytes with the FO myeloma cells
25 and keep the temperature at 37°C. Slowly add 1.5 mL PEG 1500 into the centrifuge tube within 1.5 min and shake the centrifuge tube tenderly. Thus the PEG, the splenocytes and the FO myeloma cells are mixed evenly. Leave the centrifuge tube for 1.5 min. Add 5 mL RPMI culture medium
30 into the centrifuge tube within 5 min and shake the centrifuge tube tenderly so as to mix the RPMI culture medium, the PEG, the splenocytes and the FO myeloma cells homogeneously. Then add 20 mL RPMI culture medium within 5 min. Thus the cell fusion of the splenocytes with

the FO myeloma cells is induced by PEG and occurred in the RPMI culture medium.

5 **【0037】** Next use a HT culture medium or a HAT culture medium to select the hybridoma cells fused successfully. The HT culture medium includes hypoxanthine and thymidine while the HAT culture medium consists of hypoxanthine, aminopterin and thymidine. The FO myeloma cells unfused are lack of a hypoxanthine-guanine
10 phosphoribosyl transferase (HGPRT) or a thymidine kinase (TK). Thus these FO myeloma cells are dead in the HT or HAT culture medium because they are unable to use the hypoxanthine or thymidine. Moreover, the unfused splenocytes die out in the culture medium. Thus the
15 hybridoma cells fused successfully are selected, divided into several groups and cultured respectively. Use Enzyme-linked immunosorbent assay (ELISA) to analyze the culture medium of the hybridoma cells and yield the set of the hybridoma cells able to secret antibodies against the
20 dengue antigen.

【0038】 The splenocytes and the FO myeloma cells induced by PEG in the RPMI culture medium for cell fusion are harvested by centrifugation. Remove the supernatant and
25 carefully add 45mL HT culture medium to yield a cell suspension including hybridoma cells, unfused FO myeloma cells and unfused splenocyte. The cell suspension is evenly distributed into six 96-well microplates. Each well is added with 75 μ L cell suspension and cultured at 37°C. The next
30 day add 75 μ L HAT culture medium into each well. The third day the old culture medium is replaced by 75 μ L HAT culture medium. Then replace the HAT culture medium each 3-4 days. After observing the death of FO myeloma cells used as the control set, the HAT culture medium is changed into 75

μL HT culture medium that is replaced once at the same interval (3-4days). At last, only 1 or 2 stable colony (colonies) of the hybridoma cell(s) is/are left in each well. Collect the used HT culture medium while replacing the new
5 HT culture medium and test the used HT culture medium at each well by ELISA to select the hybridoma cell(s) that secretes the antibodies against the dengue antigen.

【0039】 As to ELISA, first the purified dengue antigen is
10 coated to a 96 microwell immunoassay strip by pH 9.5 carbonate buffer. The coating is performed at 4°C overnight. Then block with 1% bovine serum albumin (BSA) at 37°C for an hour to reduce non-specific absorption of the antibodies added next. After being washed 3 times, add the
15 test sample diluted by PBST-20 (PBS buffer containing 20% Tween-20), the positive control and the negative control and vibrate at 37°C for a half hour, allowing the antibody against the dengue antigen in a part of test samples and the positive control to bind to the dengue antigen. Wash four
20 times, add Goat anti-mouse IgG-alkaline phosphatase conjugate for use as a secondary antibody and vibrate at 37°C for a half hour. Thus the secondary antibody binds to the antibody against the dengue antigen in a part of test samples and the positive control. After being washed 4
25 times, each well is added with 100 μL p-nitrophenyl phosphate (pNPP) used as coloring agent (chromogen) and is set at a dark site at room temperature for an hour to carry out coloring reaction. Next use an ELISA reader to measure the absorbance at 405 nm and 620 nm.

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【0040】 The hybridoma cells in the sets/wells that are confirmed to secrete the antibody against the dengue antigen are dispersed into a plurality of single hybridoma cell and cultured into a plurality of single strains of hybridoma cell

by limiting dilution assay. The ELISA is used again to select the hybridoma cell strains able to secrete the antibody against the dengue antigen.

- 5 **【0041】** After being confirmed by the ELISA mentioned above, the hybridoma colony/colonies that secrete/secret the antibody against the dengue antigen is/are diluted and dispersed into a 96-well microplate again. Each well includes only one hybridoma cell that grows into a colony.
- 10 Then use the above ELISA method again to screen the hybridoma cell that secretes a monoclonal antibody against the dengue antigen with higher titer. The hybridoma cell is grown in mass culture and then stored in liquid nitrogen.
- 15 **【0042】** In the step S40, the hybridoma cell is used to produce a monoclonal antibody. As mentioned above, the hybridoma cell has the capacity to produce antibody against the dengue antigen contributed by B cells (plasma cells) and the ability to form secondary cultures like tumor cells. Thus
- 20 the hybridoma cell can be cultured in vitro massively and the culture medium is collected. The hybridoma cell produces and secretes the monoclonal antibody into the culture medium. Thus the monoclonal antibody can be purified from the hybridoma cell culture medium. Moreover, the
- 25 hybridoma cell can be implanted into an experimental animal. Then the hybridoma cell produces and secretes the monoclonal antibody into a body fluid of the experimental animal. Next the monoclonal antibody can be purified from the body fluid. The experimental animal is a BALB/c mouse
- 30 and the body fluid is ascites. The hybridoma cell is injected into an abdomen of the hybridoma cell to induce a tumor. Then collect the ascites produced by the tumor. The concentration of the monoclonal antibody produced by this kind of ascites is high so that the monoclonal antibody can

be mass-produced and purified conveniently.

5 **【0043】** The ascites containing highly-concentration monoclonal antibody can be purified by a protein A affinity chromatography column that absorbs a Fc region (Fragment crystallizable region) of the monoclonal antibody. The protein A affinity chromatography column can be Bio-Rad's Affi-prep protein A cartridges.

10 **【0044】** In this embodiment, the monoclonal antibody can be used to produce a test kit for detection of dengue fever. The test kit can be a ELISA kit or an immunochromatographic test (ICT) kit. Based on fast coloring of the ELISA or ICT, the test kit that detects
15 whether a subject is infected by dengue fever or not within a short period of time is produced. The test kit can be applied to fast screening of dengue fever and used for prevention as well as control of dengue fever.

20 **【0045】** The dengue antigen(s) and the monoclonal antibody/antibodies against the dengue antigen obtained by the above steps can be tested by ELISA to determine the titer for evaluation of the feasibility of the monoclonal antibody/antibodies to develop a test kit. The test kit is
25 designed based on ELISA or ICT. The monoclonal antibody should be labeled for production of the test kit. That means the molecule of the monoclonal antibody is connected to an antibody marker so as to generate a labeled monoclonal antibody. Moreover, the antibody marker is used to carry out
30 reactions such as coloring reaction, allowing the user to learn the result of the test kit. The antibody marker can be an alkaline phosphatase (AP) or a horseradish peroxidase (HRP).

【0046】 By the production method of the monoclonal antibody revealed in the present invention, a plurality of monoclonal antibodies and a plurality of hybridoma cells are provided and used to detect the dengue fever. Use ELISA to
5 detect binding ability of the monoclonal antibody to the dengue antigen and the results are shown in table 1.

【0047】 Table 1 shows the binding strength between the tested monoclonal antibody and the NS1 antigen of four
10 dengue virus serotypes. Seven monoclonal antibodies with lab. number of NS1-5, NS1-10, NS1-11, NS1-9, NS1-3, NS1-8, NS1-4 are reacted with dengue virus type 1 NS1 antigen, dengue virus type 2 NS1 antigen, dengue virus type 3 NS1 antigen, and dengue virus type 4 NS1 antigen. The
15 plus sign represents the strength of binding while the minus sign represents there is no obvious binding.

【0048】 The monoclonal antibodies No. NS1-5, No. NS1-11, No. NS1-9, No. NS1-3, No. NS1-8, No. NS1-4 are
20 one of the IgG subclasses- IgG1 while monoclonal antibody No. NS1-10 is IgG2b.

【0049】 As shown in the table 1, the monoclonal antibody No. NS1-5 shows specific binding to the dengue virus type 1
25 NS1 antigen. The monoclonal antibody No. NS1-10 has specific binding to the dengue virus type 2 NS1 antigen. The monoclonal antibody No. NS1-11 has specific binding to the dengue virus type 3 NS1 antigen. The monoclonal antibody No. NS1-9 has specific binding to the dengue virus type 4
30 NS1 antigen. The monoclonal antibody No. NS1-3 shows binding to both dengue virus type 1 NS1 antigen and dengue virus type 2 NS1 antigen. The monoclonal antibody No. NS1-8 shows binding to dengue virus type 1 NS1 antigen, dengue virus type 2 NS1 antigen, dengue virus type 3 NS1

antigen, and dengue virus type 4 NS1 antigen. The monoclonal antibody No. NS1-4 shows binding to both dengue virus type 1 NS1 antigen and dengue virus type 3 NS1 antigen.

5

Table 1:

monoclonal antibody	type 1 dengue fever	type 2 dengue fever	type 3 dengue fever	type 4 dengue fever
NS1-5(IgG1)	+++	-	-	-
NS1-10(IgG2b)	-	++	-	-
NS1-11(IgG1)	-	-	+++	-
NS1-9 (IgG1)	-	-	-	++
NS1-3 (IgG1)	+	+++	-	-
NS1-8 (IgG1)	+++	++	+++	++
NS1-4(IgG1)	+++	-	+++	-

10 **【0050】** According to ELSIA test results, the monoclonal antibody No. NS1-5 is suitable for detection of Type 1 dengue fever. The monoclonal antibody No. NS1-10 is suitable for detection of Type 2 dengue fever. The monoclonal antibody No. NS1-11 is suitable for detection of Type 3 dengue fever. The monoclonal antibody No. NS1-9 is suitable for detection of Type 4 dengue fever.

15

【0051】 Innova Bioscience's Lightning-Link kits that

enable direct labeling of antibodies are used. The modifier of Lightning-Link modifier recognizes amino group of protein or peptide molecule and conjugates the antibody marker covalently to the protein or peptide molecule. Mix
5 100 µg monoclonal antibody with 100µg Lightning-Link-AP or 200~400 µg monoclonal antibody with 100 µg Light-Link-HRP and react at room temperature for at least 3 hours to generate the labeled monoclonal antibody. Then a quencher reagent is added to stop the reaction and
10 non-specific binding. Next 40-50% glycerol used as antifreeze is added into the labeled monoclonal antibody for frozen storage of the labeled monoclonal antibody.

【0052】 The monoclonal antibody/antibodies is/are
15 applied to the ELISA kit in the following manner. First add 100 µL, 1 µg/mL capture monoclonal antibody (without labeling) into each well of a 96-well microplate and leave to stand at 4°C overnight to allow the monoclonal antibody coating at the bottom of the 96-well microplate. Then
20 block by adding 200 µL 1% bovine serum albumin (dissolved in phosphate buffer) to each well at 37°C for an hour. After being washed by phosphate buffer, each well is added with 100 µL sample (specimen), positive control or negative control and reacted at 37°C for an hour. The dengue antigen
25 contained in a part of the samples and the positive control binds to the capture monoclonal antibody on the bottom of the 96-well microplate. Wash with phosphate buffer, add a labeled monoclonal antibody (diluted in the ratio of 1:2000), and react at 37°C for an hour. The labeled monoclonal
30 antibody binds to the dengue antigen captured by the capture monoclonal antibody. After being washed, add a substrate for the marker/label (a kind of enzyme) on the labeled monoclonal antibody and react at room temperature for 5-30 minutes. Then detect the absorbance and check which

samples include the dengue antigen according to the difference in the absorbance. Thus whether the subjects are infected or not can be learned.

5 **【0053】** While producing dengue fever ELISA kits, the monoclonal antibody No. NS1-8 is used as the capture monoclonal antibody for capturing dengue virus type 1 NS1 antigen, dengue virus type 2 NS1 antigen, dengue virus type 3 NS1 antigen, or dengue virus type 4 NS1 antigen in the
10 samples. The monoclonal antibody No. NS1-3 can also be used as the capture monoclonal antibody for capturing dengue virus type 1 NS1 antigen and dengue virus type 2 NS1 antigen in the samples. The monoclonal antibody No. NS1-4 is selected to produce the labeled monoclonal
15 antibody that recognizes the dengue virus type 1 NS1 antigen and dengue virus type 3 NS1 antigen captured by the capture monoclonal antibody. If the monoclonal antibody No. NS1-9 is selected to produce the labeled monoclonal antibody, it is applied to recognize dengue virus type 4 NS1
20 antigen captured by the capture monoclonal antibody. The dengue fever ELISA kit is tested by serum samples of a plurality of subjects. The results are shown in Fig. 2.

25 **【0054】** Refer to Fig. 2, the vertical axis represents the absorbance at 595 nm (OD595) and the horizontal axis represents the subject number. The subjects No. S1010052, No. S1010064, No. S1010145, No. S1010424 and No. S1010730 (the first to the fifth from the left) are confirmed cases of type 1 dengue fever (being infected by dengue virus
30 type 1). The subjects No. 46757, No. 47667, No. 47628, No. 47617 and No. 46336 (the sixth to the tenth from the left) are confirmed cases of type 2 dengue fever (being infected by dengue virus type 2). The subjects No. S1000711, No. S1002704, No. 45824, No. S1001057 and No. 1211 (the

eleventh to the fifteenth from the left) are confirmed cases of type 3 dengue fever (being infected by dengue virus type 3). The subjects No. 47656, No. S2117, No. S9900606, No. S9900624 and No. S9900357 (the sixteenth to the twentieth from the left) are confirmed cases of type 4 dengue fever (being infected by dengue virus type 4). The subjects No. 1S1001642, No. 1S1001643, No. 1S1001647, No. 1S1001649 and No. 1S1001650 (the twenty first to the twenty fifth from the left) are people not infected with dengue virus.

10

【0055】 As shown in Fig. 2, the dengue fever enzyme-linked immunoassay kit produced by the present invention can detect the type 1 dengue fever, type 2 dengue fever, type 3 dengue fever, and type 4 dengue fever sensitively.

15

【0056】 The monoclonal antibodies are applied to the immunochromatographic test in the following manner. The immunochromatography test kit consists of a bottom card covered by a sample pad, a particle conjugate pad, a nitrocellulose membrane and an absorbent pad in turn from one end to the other end thereof. The nitrocellulose membrane is arranged with a test line and a control line. The immunochromatography test kit is used to test a subject's sample and check whether the subject is infected with dengue virus or not.

20

25

【0057】 The sample pad is used to control flow rate of the sample and allow even distribution of the sample. The particle conjugate pad is for setting a labeled monoclonal antibody or a labeled dengue antigen (not dengue antigen). The particle conjugated pad includes a conjugated solvent for stabilizing the labeled monoclonal antibody or the labeled dengue antigen, and carrying dengue antigen and the

30

labeled monoclonal antibody in the sample or the antibody against the dengue antigen and the labeled dengue antigen in the sample to the nitrocellulose membrane. The test line and the control line on the nitrocellulose membrane are coated
5 with the dengue antigen and the monoclonal antibody against dengue respectively so as to bind to the antibody against the dengue antigen/or the labeled antibodies related to Mosquito Borne Illnesses in the sample, and the dengue antigen/or the labeled dengue antigen in the sample. The
10 nitrocellulose membrane has been treated to increase the hydrophilicity, stabilize the monoclonal antibody/ or the dengue antigen coated thereon and prevent non specific binding. The absorbent pad is used to absorb liquid in both the sample and the conjugated solvent, as well as provide
15 capillary force the sample flow required.

【0058】 The labeled monoclonal antibody can be labeled by using colloidal gold. The conjugated solvent can include high concentration cane sugar and/or other large-molecular
20 polymer used as stabilizer. The test line and the control line can be disposed on the nitrocellulose membrane by spray printing equipment. The nitrocellulose membrane is disposed over the bottom card and one end of the nitrocellulose membrane is arranged with the particle
25 conjugate pad. Then the sample pad is set over the particle conjugate pad. At last the other end of the nitrocellulose membrane is disposed with the absorbent pad. Thus a part of the particle conjugate pad is laminated to the bottom of the sample pad, a part of the nitrocellulose membrane is
30 laminated to the bottom of the particle conjugate pad, and a part of the absorbent pad is laminated to the top of the nitrocellulose membrane. The assembly of all the above components is cut after being assembled.

【0059】 The monoclonal antibodies are applied to the immunochromatographic test in a plurality of ways. There are two ways described hereinafter. First a capture monoclonal antibody is coated on the test line while the
5 particle conjugate pad is added with a labeled monoclonal antibody and a secondary antibody able to capture the labeled monoclonal antibody is coated on the control line. When the sample contains the dengue antigen, the dengue antigen binds to the labeled monoclonal antibody on the
10 particle conjugate pad. The dengue antigen is also captured by the capture monoclonal antibody on the test line. The rest labeled monoclonal antibody is moved to the control line to be captured by the secondary antibody. On the other hand, no dengue antigen is captured by the capture monoclonal
15 antibody on the test line when there is no dengue antigen in the sample. Only the labeled monoclonal antibody on the control line is captured by the secondary antibody. Thus in the positive samples (the sample contains the dengue antigen), both the test line and the control line show as a
20 colored band while only the control line is seen in negative samples (the sample contains no mosquito borne illnesses antigen).

【0060】 While producing dengue fever
25 immunochromatography test kits, both the monoclonal antibody No. NS1-8 and the monoclonal antibody No. NS1-3 can be used as the capture monoclonal antibody. The monoclonal antibody No. NS1-3 can be used for capturing dengue virus type 2 NS1 antigen in the samples while the
30 monoclonal antibody No. NS1-8 can be used for capturing dengue virus type 1 NS1 antigen, dengue virus type 2 NS1 antigen, dengue virus type 3 NS1 antigen, or dengue virus type 4 NS1 antigen in the samples. The monoclonal antibody No. NS1-4 and the monoclonal antibody No. NS1-9 are

selected to produce the labeled monoclonal antibody. The monoclonal antibody No. NS1-4 is used to recognize the dengue virus type 1 NS1 antigen and dengue virus type 3 NS1 antigen captured by the capture monoclonal antibody while the monoclonal antibody No. NS1-9 is used to recognize the dengue virus type 4 NS1 antigen. The dengue fever immunochromatography test kit produced is used to detect type 1 dengue fever, type 2 dengue fever, type 3 dengue fever, and type 4 dengue fever, as shown in Fig. 3. As mentioned above, both the test line and the control line are visible in positive samples while only the control line is seen in negative samples. The dengue fever immunochromatography test kit produced is tested by a plurality of patients' serum samples. The sensitivity of the test kit of the present invention is detected and compared with the BioRad's dengue fever immunochromatography test kit. The results are shown in table 2 and table 3.

【0061】 The table 2 shows immunochromatographic test results of NS1 antigen of four dengue virus serotypes and samples. The spiked serum samples of NS1 antigen of four dengue virus types are diluted in the ratio of 1:80, 1:160, 1:320, and 1:640 and then tested by the present invention and BioRad's immunochromatography test kit. Moreover, the serum samples of the subjects No. 47991, No. 48009, No. 48015, No. 48026, No. S10101839, No. S10101842, No. S10101892, No. S10101895, No. S10101903, No. S10101904, No. S10101905, No. S10101906, No. S10101907, No. S10101908, No. S10101909, and No. S10101910 are also detected by the present invention and BioRad's immunochromatography test kit. The subjects No. 47991, No. 48009, No. 48015, No. 48026, No. S10101839, No. S10101842, No. S10101892, and No. S10101895 are confirmed cases of dengue fever. The virus sequence in the

serum sample detected by PCR is labeled as PCR+. The NS1 in the serum sample detected is labeled as NS1+. The subjects No. S10101903, No. S10101904, No. S10101905, No. S10101906, No. S10101907, No. S10101908, No. S10101909, and No. S10101910 are not infected by dengue virus.

【0062】 Refer to table 2, it is learned that the immunochromatography test kit produced by the present invention has higher sensitivity than the immunochromatography test kit produced by BioRad company. Especially the type 4 dengue fever that is more difficult to be detected by the BioRad's immunochromatography test kit, the present invention can be used to detect the type 4 dengue fever. However, the immunochromatography test kit produced by the present invention sometimes may have false positive problem (such as the cases of S10101905 and S10101909). This can be solved by adjustment of the concentration of the serum sample.

Table 2:

Sample	Present invention	BioRad
type 1 dengue fever spiked serum (1:80)	+++	++
type 1 dengue fever spiked serum (1:160)	++	++

type 1 dengue fever spiked serum (1:320)	++	+
type 1 dengue fever spiked serum (1:640)	+	+/-
type 2 dengue fever spiked serum (1:80)	++	+
type 2 dengue fever spiked serum (1:160)	+	+
type 2 dengue fever spiked serum (1:320)	+	+/-
type 2 dengue fever spiked serum (1:640)	+/-	-
type 3 dengue fever spiked serum (1:80)	++	+
type 3 dengue fever spiked serum (1:160)	++	+/-
type 3 dengue fever spiked serum (1:320)	+	+/-
type 3 dengue fever spiked serum (1:640)	+/-	-

type 4 dengue fever spiked serum (1:80)	+	+/-
type 4 dengue fever spiked serum (1:160)	+/-	+/-
type 4 dengue fever spiked serum (1:320)	+/-	-
type 4 dengue fever spiked serum (1:640)	+/-	-
47991 (NS1+)	+++	++
48009 (PCR+/NS1-)	+	-
48015 (NS1+)	+	+/-
48026 (PCR+/NS1+)	+++	++
S10101839 (NS1+)	+	+
S10101842 (PCR+/NS1-)	+++	+++
S10101892 (PCR-/NS1+)	++	+
S10101895 (PCR+/NS1-)	++	+
S10101903	-	-
S10101904	-	-
S10101905	+	-
S10101906	-	-

S10101907	-	-
S10101908	-	-
S10101909	+	-
S101019010	-	-

【0063】 The test results of the confirmed cases of different dengue virus serotypes are shown in table 3. As shown in table 3, the serum samples of type 1 dengue fever, type 2 dengue fever, type 3 dengue fever, type 4 dengue fever, and non-typable subjects are tested by the immunochromatography test kit of the present invention and BioRad's immunochromatography test kit.

【0064】 It is learned that the sensitivity of the immunochromatography test kit produced by the present invention is higher than the sensitivity of the BioRad's immunochromatography test kit, especially the dengue virus type 4 cases or secondary infection cases that are more difficult to be detected by the BioRad's immunochromatography test kit. The immunochromatography test kit produced by the present invention has higher detection rate. This is beneficial to early diagnosis and reporting to the control bureau.

Table 3

percentage of secondary infected cases detected by the BioRad	75% (9)
percentage of primary infected cases detected by the BioRad	100% (43)
percentage of secondary infected cases detected by the present invention	91.7% (11)
percentage of primary infected cases detected by the present invention	100% (43)
secondary infection	12
Primary infection	43
Serotype	Type 1
	Type 2
	Type 3
	Type 4
	non-typable
	All

cases			(113)	(51)	(106)	(36)
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【0065】 The other way is to use binding competition among molecules. The dengue antigen is coated on the test line and the labeled monoclonal antibody is added into the particle conjugate pad. A secondary antibody able to capture the labeled monoclonal antibody is coated on the control line. When the sample contains pathogens of the mosquito borne disease, the dengue antigen binds to the labeled monoclonal antibody on the particle conjugate pad. When the labeled monoclonal antibody is moved to the test line, it is unable to bind to the dengue antigen coated on the test line. Until moved to the control line, the labeled monoclonal antibody is captured by the secondary antibody. On the other hand, the labeled monoclonal antibody doesn't bind to the dengue antigen until it is moved to the test line when the sample contains no dengue antigen. Then the rest labeled monoclonal antibody is moved to the control line and is captured by the secondary antibody. Thus only the control line is seen in the positive samples (the sample contains the dengue antigen) while both the test line and the control line are visible in the negative samples (the sample contains no dengue antigen).

【0066】 There are 7 monoclonal antibody strains selected from the monoclonal antibodies produced by the present invention and deposited at food industry research and development institute (Taiwan). The accession number of the monoclonal antibody strains is BCRC960479 (Lab No. NS1-3), BCRC960480 (Lab No. NS1-4), BCRC960481 (Lab No. NS1-5), BCRC960482 (Lab No. NS1-8), BCRC960483 (Lab No. NS1-9), BCRC960484 (Lab No. NS1-10) and BCRC960485 (Lab No. NS1-11) respectively.

【0067】 Sequence information of the monoclonal antibody are shown in table 4 and table 5; table 4 shows framework region(FR) and complementarity determining region(CDR) of heavy chain; table 5 shows framework region and complementarity determining region of light chain.

Table 4: framework region (FR) and complementarity determining region (CDR) of heavy chain.

10

	HFR1	HCDR1	HFR2	HCDR2	HFR3	HCDR3
BCRC960479	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
(NS1-3)	NO:1	NO:2	NO:3	NO:4	NO:5	NO:6
BCRC960480	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
(NS1-4)	NO:13	NO:14	NO:15	NO:16	NO:17	NO:18
BCRC960481	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
(NS1-5)	NO:25	NO:26	NO:27	NO:28	NO:29	NO:30
BCRC960482	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
(NS1-8)	NO:37	NO:38	NO:39	NO:40	NO:41	NO:42
BCRC960483	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
(NS1-9)	NO:49	NO:50	NO:51	NO:52	NO:53	NO:54
BCRC960474	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
(NS1-10)	NO:61	NO:62	NO:63	NO:64	NO:65	NO:66
BCRC960485	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
(NS1-11)	NO:73	NO:74	NO:75	NO:76	NO:77	NO:78

Table 5: framework region (FR) and complementarity determining region (CDR) of light chain.

	LFR1	LCDR1	LFR2	LCDR2	LFR3	LCDR3
BCRC960479 (NS1-3)	SEQ ID NO:7	SEQ ID NO:8	SEQ ID NO:9	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
BCRC960480 (NS1-4)	SEQ ID NO:19	SEQ ID NO:20	SEQ ID NO:21	SEQ ID NO:22	SEQ ID NO:23	SEQ ID NO:24
BCRC960481 (NS1-5)	SEQ ID NO:31	SEQ ID NO:32	SEQ ID NO:33	SEQ ID NO:34	SEQ ID NO:35	SEQ ID NO:36
BCRC960482 (NS1-8)	SEQ ID NO:43	SEQ ID NO:44	SEQ ID NO:45	SEQ ID NO:46	SEQ ID NO:47	SEQ ID NO:48
BCRC960483 (NS1-9)	SEQ ID NO:55	SEQ ID NO:56	SEQ ID NO:57	SEQ ID NO:58	SEQ ID NO:59	SEQ ID NO:60
BCRC960474 (NS1-10)	SEQ ID NO:67	SEQ ID NO:68	SEQ ID NO:69	SEQ ID NO:70	SEQ ID NO:71	SEQ ID NO:72
BCRC960485 (NS1-11)	SEQ ID NO:79	SEQ ID NO:80	SEQ ID NO:81	SEQ ID NO:82	SEQ ID NO:83	SEQ ID NO:84

5

【0068】 In summary, the present invention provides an antibody composition and a method for producing the same. The monoclonal antibody contained in the antibody composition specifically binds to dengue antigen of dengue fever for detection of serotypes of dengue fever. The dengue fever can be type 1 dengue fever, type 2 dengue fever, type 3 dengue fever, or type 4 dengue fever. The monoclonal

10

antibody is produced by a plurality of steps. First use the dengue antigen to immunize an experimental animal. Then yield a hybridoma cell by fusing a splenocyte from the experimental animal with a tumor cell. Next produce a
5 monoclonal antibody by the hybridoma cell. Therefore the antibody composition of the present invention is produced easily and applied to detect dengue fever.

【0069】 Additional advantages and modifications will
10 readily occur to those skilled in the art. Therefore, the invention in its broader aspects is not limited to the specific details, and representative devices shown and described herein. Accordingly, various modifications may be made without departing from the spirit or scope of the general
15 inventive concept as defined by the appended claims and their equivalents.

Sequence listing

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same

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1 5

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Ala Arg Asn Arg Gly Gly Tyr Pro Pro Ala Met Asp Tyr Trp

Gly Gln

25 <213> *Mus musculus*.

37

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20

25

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35

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Ile Gly Asp

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<400> 29

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Asp Thr Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu

15 20 25

Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys

30 35

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<213> *Mus musculus*.

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<400> 30

Ala Thr Tyr Tyr Gly Ser Tyr Asp Tyr Phe Asp Tyr

1 5 10

<210> 31

5 <211> 26

<212> Protein

<213> *Mus musculus*.

<400> 31

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1 5 10

Pro Gly Glu Lys Ile Thr Ile Thr Cys Ser Ala Ser

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<212> Protein

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20 <400> 32

Ser Ser Ile Ser Ser Asn Tyr

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<210> 33

25 <211> 17

<212> Protein

<213> *Mus musculus*.

<400> 33

Leu His Trp Tyr Gln Gln Lys Pro Gly Phe Ser Pro Lys Leu

1 5 10

Leu Ile Tyr

5 15

<210> 34

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10 <213> *Mus musculus*.

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25 15 20 25

Glu Asp Val Ala Thr Tyr Tyr Cys

30 35

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5 <213> *Mus musculus*.

<400> 36

Gln Gln Asp Ser Ser Ile Pro Phe Tyr

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10

<210> 37

<211> 25

<212> Protein

<213> *Mus musculus*.

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<400> 37

His Phe Tyr Leu Gln Glu Ser Gly Ala Glu Leu Val Arg Pro

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5

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Gly Ser Ser Val Lys Ile Ser Cys Lys Ala Ser

20

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25

<210> 38

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25 <213> *Mus musculus*.

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Gly Tyr Ala Phe Ser Ser Tyr Trp

1 5

<210> 39

5 <211> 17

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1 5 10

Ile Gly Gln

15

15 <210> 40

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<212> Protein

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20 <400> 40

Ile Tyr Pro Gly Asp Gly Asp Ser

1 5

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25 <211> 38

<212> Protein

<213> *Mus musculus*.

<400> 41

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1 5 10

5 Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu

15 20 25

Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys

30 35

10 <210> 42

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1 5 10

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20 <211> 26

<212> Protein

<213> *Mus musculus*.

<400> 43

25 Asp Ile Val Leu Thr Gln Thr Pro Leu Thr Leu Ser Val Thr

1 5 10

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Leu Val Ser

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<212> Protein

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<400> 47

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Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala

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<212> Protein

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Gln Gln His Tyr Ser Ser Pro Trp Thr

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<212> Protein

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 Ile Asn Pro Tyr Asn Asp Gly Thr
 1 5
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 Lys Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser
 1 5 10
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 25 Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
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Ser Ser Leu Ser Tyr

1 5

<210> 57

5 <211> 17

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1 5 10

Trp Ile Tyr

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20 <400> 58

Asp Thr Ser

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<210> 59

25 <211> 36

<212> Protein

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<400> 59

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1 5 10

5 Ser Gly Thr Ser Tyr Ser Leu Thr Ile Thr Ser Met Glu Ala

15 20 25

Glu Asp Ala Ala Thr Tyr Tyr Cys

30 35

10 <210> 60

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1 5

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20 <211> 25

<212> Protein

<213> *Mus musculus*.

<400> 61

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1 5 10

Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser
15 20 25

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5 <211> 8

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<400> 62

10 Gly Tyr Thr Phe Thr Ser Thr Trp
1 5

<210> 63

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15 <212> Protein

<213> *Mus musculus*.

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20 1 5 10
Ile Gly Tyr
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<210> 64

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Ile Asn Pro Asn Thr Gly Ser Thr

1 5

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Glu Tyr Asn Arg Glu Phe Lys Asp Lys Ala Thr Leu Thr Ala

1 5 10

Tyr Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu

15 15 20 25

Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys

30 35

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25 Ala Arg Glu Asn Tyr Arg Tyr Asp Gly Ala Met Asp Tyr

1 5 10

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<213> *Mus musculus*.

Asp Ile Val Leu Thr Gln Tyr Pro Ala Ile Leu Ser Ala Ser Pro

Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ile

15

<212> Protein

Ser Ser Val Ser Tyr

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<212> Protein

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 Ala Thr Ser
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 Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala
 15 20 25
 25 Glu Asp Ala Ala Thr Tyr Tyr Cys
 30 35

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10 <210> 73

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Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser

15

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25

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<210> 74

<211> 8

<212> Protein

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<400> 74

Ala Phe Asn Ile Lys Asp Thr Tyr

1

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<210> 75

5 <211> 17

<212> Protein

<213> *Mus musculus*.

<400> 75

10 Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp

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Ile Gly Arg

15

15 <210> 76

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20 <400> 76

Ile Asp Pro Ala Asn Asp Asn Thr

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<210> 77

25 <211> 38

<212> Protein

<213> *Mus musculus*.

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1 5 10

5 Asp Thr Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu

15 20 25

Thr Ser Asp Asp Thr Ala Val Tyr Tyr Cys

30 35

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Ala Arg Pro Ile His Tyr Tyr Gly Tyr Asp Tyr

1 5 10

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20 <211> 26

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<213> *Mus musculus*.

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1 5 10 15

Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser

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5 <211> 5

<212> Protein

<213> *Mus musculus*.

<400> 80

10 Ser Ser Ile Thr Tyr

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5

<210> 81

<211> 17

15 <212> Protein

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<400> 81

Ile His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg

20

1

5

10

Trp Ile Tyr

15

<210> 82

25 <211> 3

<212> Protein

<213> *Mus musculus*.

<400> 82

Asp Thr Ser

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<210> 83

<211> 36

<212> Protein

<213> *Mus musculus*.

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Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly

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Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala

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25

Glu Asp Ala Ala Thr Tyr Tyr Cys

30

35

<210> 84

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<211> 9

<212> Protein

<213> *Mus musculus*.

<400> 84

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Gln Gln Trp Ser Ser Ser Pro Leu Thr

1

5

Claims

What is claimed is:

1. An antibody composition comprising a first
5 monoclonal antibody, a second monoclonal
antibody, a third monoclonal antibody, and a
fourth monoclonal antibody; wherein the first
monoclonal antibody includes heavy chain
10 complementarity determining region 1(HCDR1)
of sequence ID number (SEQ ID NO):26, heavy
chain complementarity determining region
2(HCDR2) of SEQ ID NO:28, heavy chain
complementarity determining region 3(HCDR3)
15 of SEQ ID NO:30, light chain complementarity
determining region 1(LCDR1) of SEQ ID NO:32,
light chain complementarity determining region
2(LCDR2) of SEQ ID NO:34, light chain
complementarity determining region 3(LCDR3)
20 of SEQ ID NO:36; the second monoclonal
antibody includes HCDR1 of SEQ ID NO:62,
HCDR2 of SEQ ID NO:64, HCDR3 of SEQ ID
NO:66, LCDR1 of SEQ ID NO:68, LCDR2 of
SEQ ID NO:70, LCDR3 of SEQ ID NO:72; the
third monoclonal antibody includes HCDR1 of
25 SEQ ID NO:74, HCDR2 of SEQ ID NO:76,
HCDR3 of SEQ ID NO:78, LCDR1 of SEQ ID
NO:80, LCDR2 of SEQ ID NO:82, LCDR3 of
SEQ ID NO:84; the fourth monoclonal antibody
includes HCDR1 of SEQ ID NO:50, HCDR2 of
30 SEQ ID NO:52, HCDR3 of SEQ ID NO:54,
LCDR1 of SEQ ID NO:56, LCDR2 of SEQ ID
NO:58, LCDR3 of SEQ ID NO:60.

2. A detection method for detecting dengue virus
35 comprising the steps of:
coating at least one capture monoclonal antibody

at the bottom of plurality of sample wells,
wherein the at least one capture monoclonal
antibody comprises an antibody which includes
HCDR1 of SEQ ID NO:38, HCDR2 of SEQ ID
5 NO:40, HCDR3 of SEQ ID NO:42, LCDR1 of SEQ
ID NO:44, LCDR2 of SEQ ID NO:46, LCDR3 of
SEQ ID NO:48;
adding a sample, a positive control or a
negative control in the sample wells respectively
10 and then a dengue antigen in the samples and the
positive control bind to the capture monoclonal
antibody at the bottom of the sample wells;
adding at least one labeled monoclonal antibody
in the sample wells respectively and the labeled
15 monoclonal antibody bind to the dengue antigens
captured by the capture monoclonal antibodies,
wherein the labeled monoclonal antibody are an
antibody which includes HCDR1 of SEQ ID
NO:50, HCDR2 of SEQ ID NO:52, HCRD3 of
20 SEQ ID NO:54, LCDR1 of SEQ ID NO:56,
LCDR2 of SEQ ID NO:58, LCDR3 of SEQ ID
NO:60, an antibody which includes HCDR1 of
SEQ ID NO:26, HCDR2 of SEQ ID NO:28,
HCDR3 of SEQ ID NO:30, LCDR1 of SEQ ID
25 NO:32, LCDR2 of SEQ ID NO:34, LCDR3 of SEQ
ID NO:36, an antibody which includes HCDR1 of
SEQ ID NO:62, HCDR2 of SEQ ID NO:64,
HCDR3 of SEQ ID NO:66, LCDR1 of SEQ ID
NO:68, LCDR2 of SEQ ID NO:70, LCDR3 of SEQ
30 ID NO:72 or an antibody which includes HCDR1
of SEQ ID NO:74, HCDR2 of SEQ ID NO:76,
HCDR3 of SEQ ID NO:78, LCDR1 of SEQ ID
NO:80, LCDR2 of SEQ ID NO:82, LCDR3 of SEQ
ID NO:84; and
35 detecting the absorbance of the samples;
wherein the detection method is used to detect

type 1 dengue antigens, type 2 dengue antigens,
type 3 dengue antigens or type 4 dengue
antigens.

5 3. The method of claim wherein the at least one
capture monoclonal antibody further comprises an
antibody which includes HCDR1 of SEQ ID NO:2,
HCDR2 of SEQ ID NO:4, HCDR3 of SEQ ID NO:6,
LCDR1 of SEQ ID NO:8, LCDR2 of SEQ ID NO:10,
10 LCDR3 of SEQ ID NO:12.

4. A kit comprising a first monoclonal antibody, a
second monoclonal antibody, a third monoclonal
antibody, a fourth monoclonal antibody, a fifth
15 monoclonal antibody, and a sixth monoclonal
antibody, when used according to the method of
claim 2 or 3, wherein the first monoclonal
antibody includes HCDR1 of sequence ID number
(SEQ ID NO):26, HCDR2 of SEQ ID NO:28,
20 HCDR3 of SEQ ID NO:30, LCDR1 of SEQ ID
NO:32, LCDR2 of SEQ ID NO:34, LCDR3 of SEQ
ID NO:36; the second monoclonal antibody
includes HCDR1 of SEQ ID NO:62, HCDR2 of SEQ
ID NO:64, HCDR3 of SEQ ID NO:66, LCDR1 of
25 SEQ ID NO:68, LCDR2 of SEQ ID NO:70, LCDR3
of SEQ ID NO:72; the third monoclonal antibody
includes HCDR1 of SEQ ID NO:74, HCDR2 of SEQ
ID NO:76, HCDR3 of SEQ ID NO:78, LCDR1 of
SEQ ID NO:80, LCDR2 of SEQ ID NO:82, LCDR3
30 of SEQ ID NO:84; the fourth monoclonal antibody
includes HCDR1 of SEQ ID NO:50, HCDR2 of SEQ
ID NO:52, HCDR3 of SEQ ID NO:54, LCDR1 of
SEQ ID NO:56, LCDR2 of SEQ ID NO:58, LCDR3
of SEQ ID NO:60; the fifth monoclonal antibody
35 includes HCDR1 of SEQ ID NO:2, HCDR2 of SEQ
ID NO:4, HCDR3 of SEQ ID NO:6, LCDR1 of SEQ

ID NO:8, LCDR2 of SEQ ID NO:10, LCDR3 of
SEQ ID NO:12; and the sixth monoclonal antibody
specifically includes HCDR1 of SEQ ID NO:38,
HCDR2 of SEQ ID NO:40, HCDR3 of SEQ ID
5 NO:42, LCDR1 of SEQ ID NO:44, LCDR2 of SEQ
ID NO:46, LCDR3 of SEQ ID NO:48.

5. A kit comprising a first monoclonal antibody, a
second monoclonal antibody, a third monoclonal
10 antibody and a fourth monoclonal antibody, when
used according to the method of claim 2 or 3,
wherein the first monoclonal antibody includes
HCDR1 of sequence ID number (SEQ ID NO):14,
HCDR2 of SEQ ID NO:16, HCDR3 of SEQ ID
15 NO:18, LCDR1 of SEQ ID NO:20, LCDR2 of SEQ
ID NO:22, LCDR3 of SEQ ID NO:24; the second
monoclonal antibody includes HCDR1 of SEQ ID
NO:50, HCDR2 of SEQ ID NO:52, HCDR3 of SEQ
ID NO:54, LCDR1 of SEQ ID NO:56, LCDR2 of
20 SEQ ID NO:58, LCDR3 of SEQ ID NO:60; the
third monoclonal antibody includes HCDR1 of
SEQ ID NO:2, HCDR2 of SEQ ID NO:4, HCDR3 of
SEQ ID NO:6, LCDR1 of SEQ ID NO:8, LCDR2 of
SEQ ID NO:10, LCDR3 of SEQ ID NO:12; and the
25 fourth monoclonal antibody specifically includes
HCDR1 of SEQ ID NO:38, HCDR2 of SEQ ID
NO:40, HCDR3 of SEQ ID NO:42, LCDR1 of SEQ
ID NO:44, LCDR2 of SEQ ID NO:46, LCDR3 of
SEQ ID NO:48.