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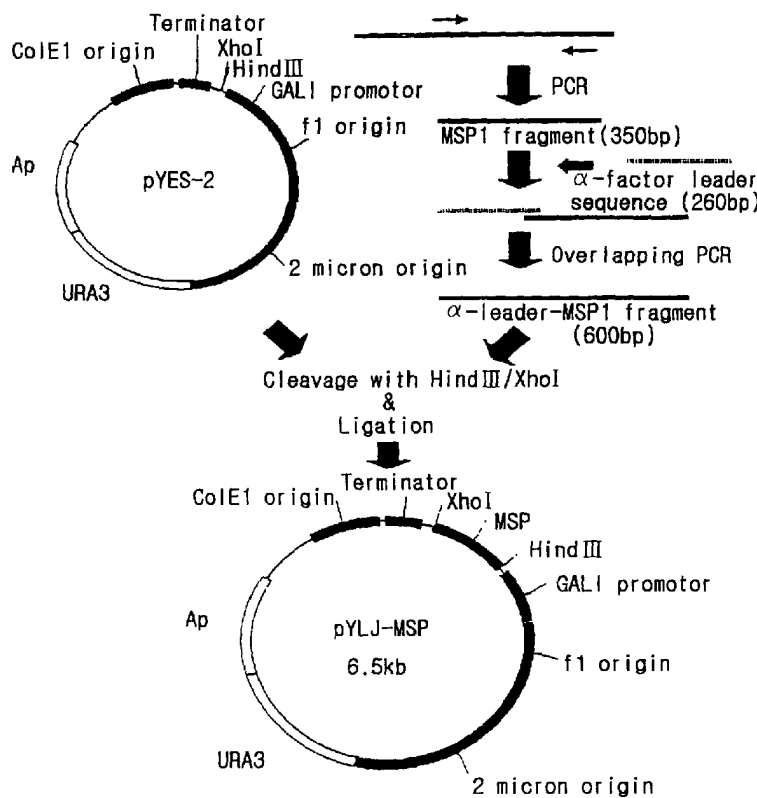
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[Continued on next page]

(54) Title: IMMUNOASSAY AND DIAGNOSTIC REAGENT FOR MALARIA



(57) Abstract: The present invention relates to an immunoassay and diagnostic reagent for malaria by using antigens of malarial Protozoa. More preferably, the present invention relates to an immunoassay and diagnostic reagent for malaria which detect malaria-specific antibodies in blood by using Merozoite Surface Protein of *Plasmodium vivax*. The immunoassay and diagnostic reagent detecting malaria-specific antibodies in blood according to the present invention have high specificity and sensitivity and are useful in diagnosing a type of malaria where latent period is long and number of Protozoa in blood is few. Also, the present invention relates to a preparation method of the surface protein of malarial Protozoa using yeast or E.Coli. Preferably, the present invention provides an expression vector comprising genes of Merozoite Surface Protein of *Plasmodium Vivax* and histidine residues, as well as transformants transformed with the expression vector. Also, the present invention provides a method for preparing Merozoite Surface Protein of malarial Protozoa by using the transformant. The surface protein Merozoite Surface Protein of malarial

Protozoa prepared from yeast or E.Coli transformant according to the present invention has high sensitivity and specificity to antibody as well as high purity. Also, the surface protein prepared by the preparation method of the present invention has markedly low pseudo-positive signals, and is useful in diagnosing malaria.



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IMMUNOASSAY AND DIAGNOSTIC REAGENT FOR MALARIA

Technical Field

The present invention relates to an immunoassay and diagnostic reagent for malaria. Particularly, the present invention relates to an immunoassay and diagnostic reagent for malaria which detects malaria-specific antibodies in blood by using antigens of malarial Protozoa. Also, the present invention relates to an immunoassay and diagnostic reagent for malaria which detects malaria-specific IgM and/or IgG in blood by using antigens of malarial Protozoa. Further, the present invention relates to a method for preparing surface protein of *Plasmodium Vivax* by using yeast or *E.Coli*.

Background Art

Malaria is a disease caused by malarial Protozoa that infects within human erythrocytes and is carried by mosquitoes. Billion people in the world reside in malaria-risk area and over 500 million people become infected by malaria each year. Malaria causes more than 2 million death each year. Malaria widely spreads all over the world, however, in some regions, malarial infection was eradicated or decreased since 1960's due to effective controls. However, recently, incidence of malaria increases all over the world again, due to increase of drug-resistant strain, increase in resistance to insecticide, abnormal weather such as El nino, etc.

Malaria can be categorized into African type, American type and Asian type. Each type differs in geographical distribution as well as characteristics in species

and genetic inclination which Protozoa exhibit.

Life cycle of malaria is divided into Schizogony and Sporogony. Schizogony is the life cycle in human host, and Sporogony is the life cycle in mosquito host. Human is infected by sporozoites by the bite of infected
5 mosquitoes. The sporozoites transfer to human liver through blood vessels, and they exist as a dormant state or multiply and develop into schizonts in hepatic cells. After a definite time, the schizonts enter the blood circulation, and multiply in full-scale. When the schizont matures, it ruptures and releases thousands of merozoites into the blood stream. Merozoites invade erythrocytes, and most merozoites go
10 through another round of asexual reproduction, again forming schizonts. Some of the merozoites change into gametocytes. The gametocytes circulate in human bloodstream. When a mosquito bites the infected person, the mosquito sucks up gametocytes along with blood. Then, male gametocyte and female gametocyte in the gut of the mosquito fuse to form zygote. The zygote, which develops in the
15 gut wall as an oocyte, eventually gives rise to the infective sporozoite, which invades the salivary glands and stomach of the mosquito. The mosquito then can infect another human host.

Drug such as chloroquine and primaquine have been used in treatment of malaria. However, recently, malaria Protozoa have developed drug resistance to
20 such Drug. Therefore, acquired drug resistance poses a serious difficulty on treatment of malaria.

In Korea, malaria has been known as Hakjil. From the 1960s, attempts to

eradicate this disease begun, and in the 1970s, cases of malaria were rarely reported. However, after a patient infected with malaria Protozoa was reported near DMZ in 1993, malaria begun to spread rapidly, and it was reported that the number of patients infected with malaria Protozoa was 3,800 in 1998.

5 Human malaria can be caused by one of four known parasites: *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale*. Among them, malaria which occurs in Korea is caused by *Plasmodium vivax* (Choi. S.Y., Korean J Parasit., 32(4), 281-284). *Plasmodium vivax* is characterized in that diagnosis thereof is difficult because latent period is long, and number of Protozoa
10 in blood is few. The imported diagnostic reagent for malaria detects malarial antigens such as Lactate Dehydrogenase of malaria. However, the imported diagnostic reagent has a difficulty in diagnosing malaria of Korean type where latent period is long, and numbers of Protozoa and antigen in blood are few, because sensitivity of the diagnostic reagent is only 50%.

15 A blood smear method has been used in the diagnosis of malaria most frequently. This method is a classical method wherein blood cells infected with malaria Protozoa are stained and the stained sample is then observed through a microscope. However, this method takes a longer time, and technical education of the examiner is required to judge blood cells infected with malaria Protozoa.
20 Recently, a diagnostic reagent detecting malarial antigens has been commercially available. However, the concurrent use of the diagnostic reagent and the blood smear method is recommended because the sensitivity of the diagnostic reagent is

low. Another method to examine genes of malaria Protozoa is Polymerase Chain Reaction method. This method has high sensitivity, however, there is a difficulty in processes thereof. Therefore, it is not put to practical use and is used only on laboratory scale. Also, such conventional diagnostic methods has a difficulty in
5 diagnosing malaria of Korean type where latent period is long, and number of Protozoa in blood is few.

Hitherto studies on expression of malarial antigen itself have been few, and methods for obtaining proteins by overexpressing genes encoding antigen proteins have been developed. Most antigens were expressed after they were fused with
10 proteins such as blood coagulation factor Xa (Ellinger et al., Virol. 180, 81, 1991), protein A of Staphylococcus(Marczinovits et al., J. Biochem., 31, 225, 1993) and β -galactosidase. As a result, expression amount of fused antigens increased. However, when they are used as diagnostic reagents, pseudo-positive signals increased due to the proteins that were partners of fusion. Although there are
15 attempts to solve the above problem by means of cutting the fused proteins(Chang et al., Biotechnology 3, 985-990, 1985 ; Ellinger et al., Virology, 180, 811-813, 1991), it is not effective in additional decrease of pseudo-positive signals, and the yield decreases and the preparation cost increases because cutting and purification processes to remove the fused proteins from the antigens are complex.

20 **Disclosure of Invention**

To solve the above-mentioned problems, the present inventors have developed an immunoassay for malaria by detecting malaria-specific antibodies in

blood of malaria patients. More particularly, the present invention relates to an immunoassay and diagnostic reagent for malaria which detects malaria-specific antibodies in blood by using antigens of malarial Protozoa. Preferably, the present invention provides an indirect immunoassay using anti-human IgG antibody and/or
5 anti-human IgM antibody, and an antigen sandwich immunoassay using a part or whole of malarial Merozoite Surface Protein(MSP).

As an another aspect, the present inventors have developed an immunoassay and diagnostic reagent for malaria which detects only malaria-specific IgM in blood of malaria patients by using antigens of malarial Protozoa. Among antibodies
10 against malaria, IgG can last for several years or several tens of years after experience of malaria. Therefore, a method of examining antibodies including IgG against malaria in the area where malaria was prevalent for a long time results in many pseudo-positive signals, which can cause confusion. In the antigen sandwich immunoassay of the present invention, few malarial antibody-positive
15 responses have been found in teens to thirties, however, 5% or more of malarial antibody-positive responses have been found in forties or more, in case of normal people uninfected with malaria. That is, relatively high IgG pseudo-positive signals occur in forties or more. This is because probability that people over forties were exposed to mosquitoes infected with malaria before 1960s is high.
20 Similarly, in the area where malaria is prevalent, malaria-specific IgGs remain in the body of man who completely recovers from malaria, and relatively high pseudo-positive signals appear in the assay for antibody to malaria. Therefore,

effectiveness of the assay for antibody against malaria to diagnose malaria will decrease. Accordingly, for diagnosing malaria in people over forties or the area where malaria is prevalent, a method of examining IgM of which titer disappears with time after the treatment of malaria will be more effective than a method of
5 examining IgG of which titer remains even after the treatment of malaria.

The immunoassay for malaria detecting malaria-specific IgM in blood by using antigens of malarial Protozoa according to the present invention is more sensitive than the prior art immunoassay detecting specific antigens, and makes an early diagnosis of malaria patient possible. Also, the immunoassay for malaria
10 detecting malaria-specific IgM according to the present invention can distinguish malaria patients from normal people who completely recover from malaria.

As an another aspect, to overcome the above-mentioned problems of the prior art in obtaining antigen proteins and develop an immunoassay and diagnostic reagent for malaria which is suitable for diagnosing malaria of Korean type, the
15 present inventors have developed a preparation method wherein erozoite Surface Protein of *Plasmodium vivax*, preferably C-terminus of Merozoite Surface Protein of *Plasmodium vivax*, more preferably polypeptide PV200C is cloned from blood of domestic malaria patients, and then is expressed from yeast or E.Coli and purified without cutting.

20 The preparation method of the present invention can produce antigens which is very sensitive to antibody as well as highly pure in a large amount by expressing the recombinant Surface Protein of malarial Protozoa from yeast or

E.Coli and then separating and purifying it in a simple method. Such produced antigens are useful in diagnostic reagents judging malaria patients by detecting antibody against malarial antigens.

In addition, such produced antigens can be used as a vaccine.

5

It is the first object of the present invention to provide an immunoassay and diagnostic reagent for malaria which detects malaria-specific antibodies in blood by using antigens of malarial Protozoa. It is the second object of the present invention to provide an indirect immunoassay using anti-human IgG antibody and/or anti-human IgM antibody as an antigen of the labeled antigen conjugate. It is the third object of the present invention to provide an antigen sandwich immunoassay using a part or whole of malarial Merozoite Surface Protein as an antigen of the labeled antigen conjugate.

It is the forth object of the present invention to provide an immunoassay and diagnostic reagent for malaria which detects malaria-specific IgM in blood by using antigens of malarial Protozoa. It is the fifth object of the present invention to provide an immunoassay and diagnostic reagent for malaria which detects malaria-specific IgM in blood by using anti-human IgM antibody.

It is the sixth object of the present invention to provide a preparation method wherein a recombinant expression vector is prepared by means of cloning genes of Merozoite Surface Protein of *Plasmodium vivax*, and is transformed into yeast and then surface protein expressed from the yeast transformant is separated

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and purified to have a high purity. It is the seventh object of the present invention to provide a method of producing antigens in a large amount for diagnosing *Plasmodium vivax*, which have high sensitivity and specificity and markedly low pseudo-positive signals by means of the above-mentioned preparation method.

5 It is the eighth object of the present invention to provide a preparation method wherein a recombinant expression vector is prepared by means of cloning genes of Merozoite Surface Protein of *Plasmodium vivax*, and is transformed into E.Coli and then surface protein expressed from the E.Coli transformant is separated and purified to have a high purity. It is the ninth object of the present invention to
10 provide a method of producing antigens in a large amount for diagnosing *Plasmodium vivax*, which have high sensitivity and specificity and markedly low pseudo-positive signals by means of the above-mentioned preparation method.

In accordance with the present invention, there are provided an immunoassay and diagnostic reagent for malaria detecting malaria-specific
15 antibodies in blood, which are more sensitive than the prior art immunoassay detecting specific antigens, and can diagnose malarial carriers as well as malaria patients, and are useful in diagnosing malaria of Korean type where latent period is long and numbers of Protozoa and antigen in blood are few.

Also, in accordance with the present invention, there are provided an
20 immunoassay and diagnostic reagent for malaria detecting malaria-specific IgM in blood which can distinguish malaria patients from normal people who completely recover from malaria.

Further, in accordance with the present invention, there is provided a preparation method wherein surface antigens of malarial Protozoa with high purity can be produced more easily and rapidly than the prior art method does. The surface protein of malarial Protozoa purified by the preparation method of the present invention has high purity, sensitivity and specificity, and markedly low pseudo-positive signals, and useful in the diagnostic reagent for malaria.

Brief Description of Drawings

Figure 1 shows a preparation method of expression vector pYLJ-MSP expressing the surface antigen of malarial Protozoa from yeast.

Figure 2 is a photograph showing a result of electrophoresis of the surface antigen purified by using Probond adsorption resin on SDS-polyacrylamide gel.

Lane 1 is the pellet after centrifugation of the cell culture;

Lane 2 is the supernatant that was concentrated after centrifugation of the cell culture; and

Lane 3 is the concentrated supernatant that was purified on Probond column.

Figure 3 is a photograph showing an assay result of the antigenicity by performing Western Blotting using the serum of the malaria patient.

Lane 4 is the cell culture that was concentrated; and

Lane 5 is the purified Merozoite Surface Protein.

Figure 4 shows a preparation method of expression vector pELK-MSP expressing PV200C polypeptide present in C-terminus of Merozoite Surface Protein of malarial Protozoa from E.Coli.

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Figure 5 is a photograph showing a result of electrophoresis of PV200C polypeptide purified by using histidine affinity resin on SDS-PAGE.

M is a size marker;

Lane 1 is the cell culture in which the expression was induced;

10

Lane 2 is the cell culture in which the expression was not induced; and

Lane 3 is the protein that was purified on a column.

Figure 6 is a photograph showing a result of Western Blotting of PV200C polypeptide using the serum of the malaria patient.

15

M is a size marker;

Lane 4 is the cell culture in which the expression was induced; and

Lane 5 is the purified Merozoite Surface Protein.

Figure 7 is a graph showing a result of diagnosing 216 malaria-positive
20 samples and 353 malaria-negative samples for malaria, according to malaria-specific IgM capture enzyme immunoassay.

Figure 8 is a graph showing results of diagnosing 75 malaria-positive samples and 92 malaria-negative samples for malaria, according to malaria-specific IgM capture enzyme immunoassay and indirect enzyme immunoassay.

5 Figure 9 is a graph showing results of diagnosing 129 malaria-negative samples classified by age for malaria, according to malaria-specific IgM capture enzyme immunoassay and antigen sandwich enzyme immunoassay.

Best Mode for Carrying out the Invention

10 To achieve the above objects, the present invention provides an immunoassay for malaria detecting malaria-specific antibodies in blood by using antigens of malarial Protozoa, and a diagnostic reagent for malaria detecting malaria-specific antibodies in blood, comprising antigens of malarial Protozoa.

Also, the present invention provides an immunoassay for malaria detecting
15 malaria-specific IgM in blood by using antigens of malarial Protozoa, and a diagnostic reagent for malaria detecting malaria-specific IgM in blood, comprising antigens of malarial Protozoa.

Preferably, the present invention provides an immunoassay for malaria detecting malaria-specific antibodies in blood, comprising (a) immobilizing surface
20 antigens of malarial Protozoa on a solid support; (b) pouring blood serum or plasma sample to combine malaria-specific antibodies with the antigens immobilized on the solid support; (c) adding labeled antigen conjugates consisting of antigens and

markers to combine the labeled antigen conjugates with the malaria-specific antibodies; and (d) analyzing by using the markers of the labeled antigen conjugates bound to the malaria-specific antibodies.

Preferably, the present invention provides an IgM capture immunoassay for
5 malaria detecting malaria-specific IgM in blood, comprising : (i) immobilizing anti-human IgM antibodies on a solid support; (ii) pouring blood serum or plasma sample to combine malaria-specific IgM with the anti-human IgM antibodies immobilized on the solid support; (iii) adding labeled antigen conjugates consisting of markers and antigens of malarial Protozoa to combine the labeled antigen
10 conjugates with the malaria-specific IgM; and (iv) analyzing by using the markers of the labeled antigen conjugates bound to the malaria-specific IgM.

Preferably, the present invention provides a diagnostic reagent for malaria detecting malaria-specific antibodies in blood, comprising a solid support coated with surface antigens of malarial Protozoa; labeled antigen conjugates consisting of
15 antigens and markers; and a substrate solution containing a color fixing agent.

Preferably, the present invention provides a diagnostic reagent for malaria detecting malaria-specific IgM in blood, comprising : a solid support coated with anti-human IgM antibodies; labeled antigen conjugates consisting of markers and antigens of malarial Protozoa; and a substrate solution containing a color fixing
20 agent.

Also, the present invention provides an expression vector comprising genes of Merozoite Surface Protein of *Plasmodium Vivax*, α -factor leader peptide of yeast

and histidine residues. The present invention provides a yeast transformant pYLJ-MSP/*S. cerevisiae* INVSC1 (Deposit No. KCTC 0937BP) transformed with the above expression vector. The present invention provides a method for preparing Merozoite Surface Protein of malarial Protozoa by using a yeast transformant. The present invention provides a diagnostic reagent for malaria, comprising the surface proteins produced by the above preparation method.

Further, the present invention provides an expression vector comprising genes of Merozoite Surface Protein of *Plasmodium Vivax*, histidine marker and T7 promotor. The present invention provides E. Coli transformant pELK-MSP/BL21 (Deposit No. KCTC 0936BP) transformed with the above expression vector. The present invention provides a method for preparing Merozoite Surface Protein of malarial Protozoa by using E. Coli transformant. The present invention provides a diagnostic reagent for malaria, comprising the surface proteins produced by the above preparation method.

15

The present invention will hereinafter be described in more detail.

The present invention provides an immunoassay for malaria detecting malaria-specific antibodies in blood by using antigens of malarial Protozoa, and a diagnostic reagent for malaria detecting malaria-specific antibodies in blood, comprising antigens of malarial Protozoa. Also, the present invention provides an immunoassay for malaria detecting malaria-specific IgM in blood by using antigens of malarial Protozoa, and a diagnostic reagent for malaria detecting malaria-specific

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IgM in blood, comprising antigens of malarial Protozoa.

Preferably, in the above immunoassay and diagnostic reagents of the present invention, a recombinant antigen may be used as the antigen of malarial Protozoa.

Preferably, in the above immunoassay and diagnostic reagents of the present invention, a surface antigen of malarial Protozoa may be used as the antigen of malarial Protozoa. A preferable example of the surface antigen of malarial Protozoa includes a part or whole of Merozoite Surface Protein of *Plasmodium Vivax*, and a more preferable example includes C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*. The above C-terminus of Merozoite Surface Protein of *Plasmodium Vivax* comprises amino acid sequences that are common to subspecies of *Plasmodium Vivax*, and especially comprises a polypeptide consisting of 108 amino acids (referred as "PV200C" hereafter) which exhibit 100% homology among subspecies of *Plasmodium Vivax*, as shown in SEQ. ID NO : 1. A preferable example of the C-terminus of Merozoite Surface Protein of *Plasmodium Vivax* includes a part or whole of PV200C polypeptide in the C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*. Also, a preferable example of amino acid sequences of the PV200C polypeptide includes amino acid sequences as shown in SEQ. ID NO : 1.

The PV200C polypeptide can be expressed in a large amount from recombinant transformants. The present inventors prepared a yeast transformant and E.Coli transformant which express PV200C polypeptide, and deposited them in Korean Collection for Type Cultures of Korea Research Institute of Bioscience and

Biotechnology on December 18, 1999 (Deposit No. KCTC 0937BP, KCTC 0936BP).

The present invention provides an immunoassay for malaria detecting malaria-specific antibodies in blood by using antigens of malarial Protozoa. Preferably, the above immunoassay detecting malaria-specific antibodies in blood according to the present invention maybe an enzyme immunoassay.

Preferably, the present invention provides an immunoassay for malaria detecting malaria-specific antibodies in blood, comprising (a) immobilizing surface antigens of malarial Protozoa on a solid support; (b) pouring blood serum or plasma sample to combine malaria-specific antibodies with the antigens immobilized on the solid support; (c) adding labeled antigen conjugates consisting of antigens and markers to combine the labeled antigen conjugates with the malaria-specific antibodies; and (d) analyzing by using the markers of the labeled antigen conjugates bound to the malaria-specific antibodies.

Preferably, in the above immunoassay, the present invention provides an indirect immunoassay for malaria wherein the antigen of the labeled antigen conjugate in the step (c) is anti-human IgG antibody and/or anti-human IgM antibody.

Preferably, in the above immunoassay, the present invention provides an antigen sandwich immunoassay for malaria wherein the antigen of the labeled antigen conjugate in the step (c) is a surface antigen of malarial Protozoa. A preferable example of the surface antigen of malarial Protozoa includes a part or

whole of Merozoite Surface Protein of *Plasmodium Vivax*, and a more preferable example includes C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*. A preferable example of the C-terminus of Merozoite Surface Protein of *Plasmodium Vivax* includes a part or whole of PV200C polypeptide in the C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*. Also, a preferable example of amino acid sequences of the PV200C polypeptide includes amino acid sequences as shown in SEQ. ID NO : 1.

Also, as a preferable example of the above immunoassay for malaria, the present invention provides an immunoassay detecting malaria-specific antibodies in blood, comprising :

- (a') expressing Merozoite surface antigens of *Plasmodium Vivax* from transformants and purifying it;
- (a) immobilizing the purified surface antigens on a solid support;
- (b) pouring blood serum or plasma sample to combine malaria-specific antibodies with the surface antigens immobilized on the solid support;
- (c) adding labeled antigen conjugates consisting of antigens and markers to combine the labeled antigen conjugates with the malaria-specific antibodies;
- (d) inducing color development by using the markers of the labeled antigen conjugates bound to the malaria-specific antibodies; and
- (e) measuring the absorbance by using a 96-well plate reader.

A preferable example of the above solid support includes wells of a well

plate. Also, a preferable example of the above method may further comprise a step of washing the solid support such as well plate after the step of combining the labeled antigen conjugate with the malaria-specific antibodies. The step of analyzing by using the markers of the labeled antigen conjugates includes, for example, the step of inducing color development by using the markers of the labeled antigen conjugates bound to the malaria-specific antibodies; and the step of measuring the absorbance by using a 96-well plate reader. The step of inducing color development by using the markers of the labeled antigen conjugates includes, for example, addition of a substrate solution consisting of a buffer solution and a color fixing agent.

As one embodiment, the present invention provides an indirect immunoassay for malaria detecting malaria-specific antibodies in blood, using a solid support coated with antigens of malarial Protozoa, preferably surface antigens of malarial Protozoa. The above immunoassay uses anti-human IgG antibody and/or anti-human IgM antibody derived from goat combined with marker, preferably horse radish peroxidase(HRP) as a labeled antigen conjugate.

In the embodiment of indirect immunoassay for malaria of the present invention, malaria-specific antibodies in blood of the patient are combined with the surface antigens of malarial Protozoa immobilized on the solid support and then HRP-anti-human IgG antibody or HRP-anti-human IgM antibody which is the labeled antigen conjugate is combined with the antigen-antibody complex. Thereafter, a color fixing agent that is included in the substrate solution added is

decomposed by HRP of the labeled antigen conjugate to induce a color development, and then absorbance is measured by using a 96-well plate reader to detect existence of malaria-specific antibodies and amount of the antibodies. In the above indirect immunoassay, malaria-positive samples(plasma of the malaria patients) and malaria-negative samples(plasma of normal people) were diagnosed. As a result, the above method exhibited approximately 90% of sensitivity and 95.8% of specificity.

As another embodiment, the present invention provides an antigen sandwich immunoassay for malaria detecting malaria-specific antibodies in blood, using a solid support coated with antigens of malarial Protozoa, preferably surface antigens of malarial Protozoa. The above immunoassay uses a surface antigen of malarial Protozoa, preferably a part or whole of Merozoite Surface Protein of *Plasmodium Vivax* combined with marker, preferably horse radish peroxidase(HRP) as a labeled antigen conjugate.

In the embodiment of antigen sandwich immunoassay for malaria of the present invention, malaria-specific antibodies in blood of the patient are combined with the surface antigens of malarial Protozoa immobilized on the solid support, and then HRP-surface antigen of malarial Protozoa which is the labeled antigen conjugate is combined with the antigen-antibody complex. Thereafter, a color fixing agent that is included in the substrate solution added is decomposed by HRP of the labeled antigen conjugate to induce a color development, and then absorbance is measured by using a 96-well plate reader to detect existence of malaria-specific

antibodies and amount of the antibodies. In the above antigen sandwich immunoassay, malaria-positive samples(plasma of the malaria patients) and malaria-negative samples(plasma of normal people) were diagnosed. As a result, the above method exhibited 98.5% of sensitivity and 99.6% of specificity.

5 The above indirect immunoassay indirectly detects malaria-specific antibodies in blood of the patient, by using anti-human IgG antibody and/or anti-human IgM antibody derived from goat as an antigen of the labeled antigen conjugate. In the meantime, the above antigen sandwich immunoassay can detect all malaria-specific antibodies in blood of the patient, by using a surface antigen
10 itself derived from malarial Protozoa as an antigen of the labeled antigen conjugate.

The immunoassay for malaria of the present invention provides a solid support coated with antigens of malarial Protozoa. When the blood sample of man who is suspected to be a malaria patient is poured into the solid support, malaria-specific antibodies in blood become specifically combined with the antigens
15 immobilized on the solid support. After the labeled antigen conjugate is added to this, the labeled antigen conjugate becomes combined with malaria-specific antibodies by recognizing Fc (fragment crystallization) region of the malaria-specific antibodies combined with the antigens immobilized on the solid support. Then, the marker of the labeled antigen conjugate decomposes the color fixing
20 agent in the substrate solution to induce a color development. Finally, the immunoassay for malaria of the present invention diagnose the infection of malaria by detecting existence of antibodies in blood and amount of the antibodies by

measuring the absorbance.

The above indirect immunoassay of the present invention detects malaria-specific antibodies combined with the antigen immobilized on the solid support by using marker-anti-human IgG antibody and/or marker-anti-human IgM antibody.

5 Also, the above antigen sandwich immunoassay of the present invention detects malaria-specific antibodies combined with the antigen immobilized on the solid support by using the labeled antigen conjugate comprising the antigen of malarial Protozoa. In above two methods, plasma of the malaria patients and plasma of normal people were diagnosed for malaria. As a result, both methods exhibited
10 more than 90 % of sensitivity and specificity.

The present invention provides a diagnostic reagent for malaria detecting malaria-specific antibodies in blood, comprising a solid support coated with surface antigens of malarial Protozoa; labeled antigen conjugates consisting of antigens and markers; and a substrate solution containing a color fixing agent.

15 A preferable example of the surface antigen of malarial Protozoa includes a part or whole of Merozoite Surface Protein of *Plasmodium Vivax*, and a more preferable example includes C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*. A preferable example of the C-terminus of Merozoite Surface Protein of *Plasmodium Vivax* includes a part or whole of PV200C polypeptide in
20 the C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*. Also, a preferable example of amino acid sequences of the PV200C polypeptide includes amino acid sequences as shown in SEQ. ID NO : 1.

Preferably, in the above diagnostic reagent, the present invention may utilize anti-human IgG antibody and/or anti-human IgM antibody as the antigen of the labeled antigen conjugate.

Preferably, in the above diagnostic reagent, the present invention may utilize
5 a surface antigen of malarial Protozoa as the antigen of the labeled antigen conjugate. A preferable example of the surface antigen of malarial Protozoa includes a part or whole of Merozoite Surface Protein of *Plasmodium Vivax*, and a more preferable example includes C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*. A preferable example of the C-terminus of Merozoite Surface
10 Protein of *Plasmodium Vivax* includes a part or whole of PV200C polypeptide in the C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*. Also, a preferable example of amino acid sequences of the PV200C polypeptide includes amino acid sequences as shown in SEQ. ID NO : 1.

Preferably, the present invention may utilize an enzyme such as horse radish
15 peroxidase(HRP) and alkaline phosphatase, colloidal gold, fluorescent materials, dyes or the like as the marker of the labeled antigen conjugate. More preferably, the present invention may utilize horse radish peroxidase(HRP) as the marker of the labeled antigen conjugate.

In the above diagnostic reagent of the present invention, the labeled antigen
20 conjugate detects existence of malaria-specific antibodies by binding to Fc (fragment crystallization) region of the malaria-specific antibodies in which there is little variation.

As a preferable labeled antigen conjugate, the present invention provides HRP-anti-human IgG antibody and/or HRP-anti-human IgM antibody wherein anti-human IgG antibody and/or HRP-anti-human IgM antibody is/are derived from goat. Also, as a preferable labeled antigen conjugate, the present invention provides HRP-
5 surface antigen conjugate wherein the surface antigen is a part or whole of surface protein of Malaria Protozoa.

The diagnostic reagent of the present invention comprises a substrate solution inducing color development when reacted with the marker of the labeled antigen conjugate. Preferably, the substrate solution consists of a buffer solution
10 and a color fixing agent which induces color development when reacted with the marker of the labeled antigen conjugate. A preferable example of the color fixing agent includes tetramethylbenzidine and the like. The color fixing agent such as tetramethylbenzidine in the substrate solution is decomposed by HRP which is a preferable marker of the labeled antigen conjugate to induce a color development.
15 Then, existence of malaria-specific antibodies and amount of the antibodies are detected by measuring the absorbance.

The present invention provides an immunoassay for malaria detecting malaria-specific IgM in blood by using antigens of malarial Protozoa. Preferably,
20 the present invention provides an IgM capture immunoassay detecting malaria-specific IgM in blood by using antigens of malarial Protozoa, and more preferably, the present invention provides an IgM capture enzyme immunoassay detecting

malaria-specific IgM in blood by using antigens of malarial Protozoa. Also, preferably, the present invention provides an immunoassay detecting malaria-specific IgM in blood by using antigens of malarial Protozoa and anti-human IgM antibody.

5 The present invention provides a 96-well plate coated with anti-human IgM antibody derived from goat as a preferable solid support for diagnosing malaria. Also, the present invention provides a labeled antigen conjugate consisting of a marker and an antigen of malarial Protozoa to detect malaria-specific IgM. The labeled antigen conjugate of the present invention consists of a marker and an
10 antigen, and the antigen maybe preferably a recombinant surface antigen of malarial Protozoa purified from yeast or E.Coli transformant to detect malaria-specific IgM. The marker of the labeled antigen conjugate includes, but is not limited to, for example, an enzyme such as horse radish peroxidase(HRP) and alkaline phosphatase, colloidal gold, fluorescent materials, dyes or the like. Preferably, the
15 marker of the labeled antigen conjugate is an enzyme, and more preferably, horse radish peroxidase(HRP).

A preferable example of IgM capture immunoassay for malaria detecting malaria-specific IgM in blood according to the present invention, comprises (i)
20 immobilizing anti-human IgM antibodies on a solid support; (ii) pouring blood serum or plasma sample to combine malaria-specific IgM with the anti-human IgM antibodies immobilized on the solid support; (iii) adding labeled antigen conjugates

consisting of markers and antigens of malarial Protozoa to combine the labeled antigen conjugates with the malaria-specific IgM; and (iv) analyzing by using the markers of the labeled antigen conjugates bound to the malaria-specific IgM.

Preferably, in the above IgM capture immunoassay, the present invention
5 may utilize a surface antigen of malarial Protozoa as the antigen of the labeled antigen conjugate in the step (iii). A preferable example of the surface antigen includes a part or whole of Merozoite Surface Protein of *Plasmodium Vivax*, and a more preferable example includes C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*. A preferable example of the C-terminus of Merozoite Surface
10 Protein of *Plasmodium Vivax* includes a part or whole of PV200C polypeptide in the C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*. Also, a preferable example of amino acid sequences of the PV200C polypeptide includes amino acid sequences as shown in SEQ. ID NO : 1.

A more preferable example of IgM capture immunoassay for malaria
15 detecting malaria-specific IgM in blood according to the present invention, comprises (1) pouring blood serum or plasma sample into each well of the well plate coated with anti-human IgM antibody derived from goat; (2) washing the well plate; (3) adding the labeled antigen conjugate consisting of HRP and the recombinant surface antigen to combine the labeled antigen conjugate with the IgM
20 bound to the anti-human IgM antibody; (4) washing the well plate; (5) adding a substrate solution consisting of a buffer solution and a color fixing agent to induce color development; and (6) measuring the absorbance by using a 96-well plate

reader.

As a preferable embodiment, the immunoassay for malaria of the present invention provides a solid support coated with anti-human IgM antibody derived from goat. Also, as a preferable embodiment, the immunoassay for malaria of the present invention provides a labeled antigen conjugate wherein an antigen protein is bound to HRP as described in the example 3-3 below. The antigen protein is expressed from the recombinant yeast or E.Coli which is transformed with the expression vector prepared by insertion of genes encoding antigen proteins of *Plasmodium vivax*, and purified.

10

When the blood sample of man who is suspected to be a malaria patient is poured into the solid support coated with anti-human IgM antibody derived from goat, human IgM in blood becomes combined with the anti-human IgM antibody immobilized on the solid support. After the labeled antigen conjugate consisting of HRP and surface antigen of malarial Protozoa is added to the solid support, the conjugate becomes combined with IgM of the malaria patient among IgMs bound to the anti-human IgM antibodies immobilized on the solid support. The HRP of the conjugate bounded to IgM of the malaria patient decomposes the color fixing agent in the substrate solution to induce color development. Finally, the immunoassay for malaria of the present invention diagnoses the infection of malaria by detecting existence of the IgM in blood and amount of the IgM by measuring the absorbance.

20

In the above immunoassay of the present invention, plasmas of the malaria

patients and plasmas of normal people were diagnosed for malaria. As a result, the above method exhibited more than 98% of sensitivity and more than 99% of specificity. Particularly, all the plasmas of normal people in forties or more who were infected with malaria in the past and are suspected to have IgG against malaria
5 were diagnosed to be negative.

The present invention provides a diagnostic reagent for malaria detecting malaria-specific IgM in blood, comprising antigens of malarial Protozoa. Preferably, the present invention provides a diagnostic reagent for malaria detecting malaria-specific IgM in blood, comprising a solid support coated with anti-human
10 IgM antibodies; labeled antigen conjugates consisting of markers and antigens of malarial Protozoa; and a substrate solution containing a color fixing agent.

Preferably, in the above diagnostic reagent, the present invention may utilize a surface antigen of malarial Protozoa as the antigen of the labeled antigen conjugate. A preferable example of the surface antigen of malarial Protozoa
15 includes a part or whole of Merozoite Surface Protein of *Plasmodium Vivax*, and a more preferable example includes C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*. A preferable example of the C-terminus of Merozoite Surface Protein of *Plasmodium Vivax* includes a part or whole of PV200C polypeptide in the C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*. Also, a
20 preferable example of amino acid sequences of the PV200C polypeptide includes amino acid sequences as shown in SEQ. ID NO : 1.

Anti-human IgM antibody for the diagnostic reagent of the present invention

includes, but is not limited to, for example, anti-human IgM antibody derived from goat. Preferably, the anti-human IgM antibody is specific to mu chain of IgM, purified by affinity between antigen and antibody, and does not cross-react with IgG or other immunoglobulin.

5 The marker of the labeled antigen conjugate for the diagnostic reagent of the present invention includes, but is not limited to, for example, an enzyme such as horse radish peroxidase(HRP) and alkaline phosphatase, colloidal gold, fluorescent materials, dyes or the like. Preferably, the marker of the labeled antigen conjugate is horse radish peroxidase(HRP).

10 The diagnostic reagent of the present invention comprises a substrate solution inducing color development when reacted with the marker of the labeled antigen conjugate. Preferably, the substrate solution consists of a buffer solution and a color fixing agent which induces color development when reacted with the marker of the labeled antigen conjugate. A preferable example of the color fixing
15 agent includes tetramethylbenzidine and the like. The color fixing agent such as tetramethylbenzidine in the substrate solution is decomposed by HRP which is a preferable marker of the labeled antigen conjugate to induce color development. Then, existence of the IgM and amount of the IgM are detected by measuring the absorbance.

20

The present invention provides an expression vector comprising genes of Merozoite Surface Protein of *Plasmodium Vivax*, α -factor leader peptide of yeast

and histidine residues. Preferably, the genes of Merozoite Surface Protein in the expression vector of the present invention may comprise a part or whole of genes of Merozoite Surface Protein of *Plasmodium Vivax*. The genes of PV200C polypeptide present in the C-terminus of Merozoite Surface Protein of *Plasmodium*
5 *Vivax* are preferable as the part or whole of genes of Merozoite Surface Protein of *Plasmodium Vivax*. PV200C polypeptide is known to have amino acid sequences that are common to several subspecies of *Plasmodium Vivax*. More preferably, in the expression vector of the present invention, amino acid sequences of the PV200C polypeptide are amino acid sequences as shown in SEQ. ID NO : 1.

10 Preferably, the present invention provides an expression vector pYLJ-MSP having a cloning map(or restriction enzyme site map) as shown in Figure 1. Also, the present invention provides a yeast transformant pYLJ-MSP/*S. cerevisiae* INVSC1 (Deposit No. KCTC 0937BP) transformed with the above expression vector. pYLJ-MSP comprises α -factor leader peptide of yeast linked with N-
15 terminus of PV200C polypeptide, PV200C polypeptide and six histidine residues linked with C-terminus of PV200C polypeptide.

To clone the PV200C polypeptide, the present inventors purified RNA from malaria-positive blood, and prepared cDNA from the above RNA. Thereafter, the present inventors obtained amplified DNA fragments of PV200C by performing
20 polymerase chain reaction(PCR) by using a pair of primers specific to PV200C and using the above cDNAs as a template.

In the meantime, to obtain proteins glycosylated in an active form by

making PV200C polypeptide expressed in the yeast to be secreted into out of cell, α -factor leader peptide sequence of yeast was linked with N-terminus of PV200C polypeptide. Amplified DNA fragments of the α -factor leader peptide sequence of yeast could be obtained by performing polymerase chain reaction(PCR) using
5 plasmids containing the α -factor leader peptide sequence of yeast.

To link the DNA fragments of the α -factor leader peptide of yeast with genes of PV200C polypeptide obtained as described above, an overlapping PCR was performed using the two PCR products as templates. As a result, it was found that the genes consisted of genes encoding 108 amino acids, as shown in SEQ. ID NO :

10 1. The present inventors obtained pYLJ-MSP which is a recombinant expression vector by inserting fragments of the genes into pYES-2 vector (refer to Figure 1). Also, the present inventors obtained a transformant pYLJ-MSP/*S. cerevisiae* INVSC1 by transforming a yeast with the recombinant expression vector pYLJ-MSP, and deposited the transformant in Korean Collection for Type Cultures of
15 Korea Research Institute of Bioscience and Biotechnology deposited on December 18, 1999 (Deposit No. KCTC 0937BP).

Also, the present invention provides a method for preparing Merozoite Surface Protein of malarial Protozoa by using a yeast transformant. Further, the present invention provides a diagnostic reagent for malaria, comprising the surface
20 proteins produced by the preparation method. Preferably, the present invention provides a method for preparing Merozoite Surface Protein of malarial Protozoa by using a yeast transformant, comprising (i) preparing an expression vector of

Merozoite Surface Protein of malarial Protozoa; (ii) transforming the expression vector into a yeast to obtain a yeast transformant; (iii) culturing the yeast transformant to obtain surface proteins; and (iv) separating and purifying the surface proteins. More preferably, in the above preparation method, the present invention
5 may use a column having an affinity for histidine and gel filtration chromatography as the step (iv). Preferably, the Merozoite Surface Protein of malarial Protozoa may be PV200C polypeptide in an active form. The PV200C polypeptide expressed from the above transformant was secreted into out of cell by the α -factor leader peptide coupled with N-terminus of PV200C. After secretion, the α -factor
10 leader peptide was removed by peptidase existing in the cell membrane. Accordingly, it was confirmed that only the surface protein was secreted into culture medium.

Because the surface protein expressed from the yeast transformant was secreted into culture medium, the present inventors separated the surface protein by
15 adsorbing the surface protein on Probond column by utilizing six histidine residues linked with C-terminus of the surface protein. Then, Merozoite Surface Protein having 99% or more of high purity was obtained by purifying through a gel filtration chromatography(refer to Figure 2). The separated and purified Merozoite Surface Protein has approximately 18 KDa, and it was confirmed that the expressed
20 surface protein was PV200C polypeptide of malarial Protozoa by analysis of amino acid sequences in N-terminus of the surface protein.

In the meantime, Western Blotting was performed with the serum of malaria

patients to assay antigenicity of the surface protein separated and purified from the yeast transformant. As a result, only MSP PV200C polypeptide having 18 KDa was detected(refer to Figure 3). Therefore, it was proved that the protein separated and purified from the yeast transformant according to the preparation method of the present invention was an antigen having high sensitivity and specificity.

The present invention provides an expression vector comprising genes of Merozoite Surface Protein of *Plasmodium Vivax*, histidine marker and T7 promotor. Preferably, the genes of Merozoite Surface Protein in the expression vector of the present invention may comprise a part or whole of genes of Merozoite Surface Protein of *Plasmodium Vivax*. The genes of PV200C polypeptide in the C-terminus of Merozoite Surface Protein of *Plasmodium Vivax* are preferable as the part or whole of genes of Merozoite Surface Protein of *Plasmodium Vivax*. More preferably, in the expression vector of the present invention, amino acid sequences of the PV200C polypeptide are amino acid sequences as shown in SEQ. ID NO : 1. Preferably, the present invention provides an expression vector pELK-MSP having a cleavage map as shown in Fig. 4. Also, the present invention provides E. Coli transformant pELK-MSP/BL21 (Deposit No. KCTC 0936BP) transformed with the above expression vector. In a preferable embodiment, the present invention utilizes genes having a size of approximately 330 bp, which are obtained by polymerase chain reaction(PCR) from blood of malaria patients. It is apparent to those skilled in the art that size of genes to be inserted into adventitious gene

insertion section of the expression vector, base pair sequence and the like may be varied by prior art techniques.

To obtain the expression vector which can produce the PV200C polypeptide in a large amount from E.Coli, pELK-MSP is prepared by cloning genes of PV200C in downstream of T7 promotor of pET-19b(Refer to Figure 4). Because there are lacZ genes in pET-19b that is a starting vector, when isopropyl thio- β -D-galactoside (IPTG) is added, IPTG acts as an expression inducer and induce the expression of lacZ. Therefore, the expression of PV200C from T7 promotor becomes induced.

Also, 10 histidine residues which do not make pseudo-positive signals are expressed in a state where they are fused with N-terminus of PV200C polypeptide expressed by the expression vector. Therefore, the PV200C polypeptide expressed by the expression vector is characterized in that it can be easily purified by histidine affinity resin. When the recombinant proteins were expressed in E.Coli, it was confirmed that the yield was 15~20% of the total proteins.

Further, the present invention provides E.Coli transformed with the pELK-MSP. The E.Coli transformant can be prepared by introducing pELK-MSP into E.Coli strain, BL21(DE3)lysS. The introduction method may be a prior art method such as heat shock method and electroporation. Besides BL21(DE3)lysS, various E.Coli strains for transformation can be used.

E.Coli/BL21(DE3)lysS/pELK-MSP was deposited in Korean Collection for Type Cultures of Korea Research Institute of Bioscience and Biotechnology deposited on December 18, 1999 (Deposit No. KCTC 0936BP).

Also, the present invention provides a method for preparing Merozoite Surface Protein of malarial Protozoa by using E.Coli transformant. Further, the present invention provides a diagnostic reagent for malaria, comprising the surface proteins produced by the preparation method.

5 Preferably, the present invention provides a method for preparing Merozoite Surface Protein of malarial Protozoa by using E. Coli transformant, comprising (i) preparing an expression vector of Merozoite Surface Protein of malarial Protozoa; (ii) transforming the expression vector into E. Coli to obtain E. Coli transformant; (iii) culturing the E. Coli transformant to obtain surface proteins; and (iv) separating
10 and purifying the surface proteins. More preferably, in the above preparation method, the present invention may use a column having an affinity for histidine and gel filtration chromatography as the step (iv). Preferably, the Merozoite Surface Protein of malarial Protozoa may be PV200C polypeptide in an active form. Because 10 histidine residues are expressed in a state where they are linked with N-
15 terminus of PV200C polypeptide expressed by the expression vector, the PV200C polypeptide expressed by the expression vector is firstly purified by histidine affinity resin. After the separated protein is concentrated, the protein is secondly purified by continuously performing gel filtration chromatography. It was confirmed that antigens of malarial Protozoa having 99% or more of high purity can
20 be obtained by the above method.

The PV200C protein obtained by the above purification method consists of 108 amino acids, has a size of 15 KDa and polypeptides that are not severed(refer to

Figure 5). Antigenicity and sensitivity of the above antigen were examined using the serum of malaria patient. As a result, it was confirmed by Western Blotting that the malaria patient had antibodies against PV200C and the sensitivity thereof was very high. Therefore, the above antigen can be used in a diagnostic reagent(refer to Figure 6).

The previously described versions of the present invention include many advantages as follows.

The immunoassay and diagnostic reagent for malaria detecting malaria-specific antibodies in blood according to the present invention are more sensitive than the prior art immunoassay detecting specific antigens, and can diagnose malarial carriers as well as malaria patients, and are useful in diagnosing malaria of Korean type where latent period is long, numbers of Protozoa and the antigen in blood are few, and the probability of recurrence is high.

The indirect immunoassay using anti-human IgG antibody and/or anti-human IgM antibody and the antigen sandwich immunoassay using the labeled antigen conjugate consisting of the marker and surface antigen, which are embodiments of the present invention, can diagnose even malarial carriers with high sensitivity, because they are methods detecting malarial-specific antibodies. Also, the indirect immunoassay and the antigen sandwich immunoassay of the present invention can make analysis simple, because blood samples and the labeled antigen conjugates were reacted simultaneously.

Further, the diagnostic reagent for malaria according to the present invention, comprising a solid support coated with surface antigens of malarial Protozoa; labeled antigen conjugates consisting of antigens and markers; and a substrate solution containing a color fixing agent, can detect existence of malaria-specific antibodies in blood and amount of the antibodies more simply and precisely.

The immunoassay and diagnostic reagent for malaria detecting malaria-specific IgM in blood by using antigens of malarial Protozoa according to the present invention can distinguish malaria patients from normal people who completely recover from malaria, because they detect only malaria-specific IgM in blood. Therefore, malaria can be diagnosed effectively in older people and in the area where malaria was prevalent for a long time.

Also, the immunoassay and diagnostic reagent for malaria detecting malaria-specific IgM in blood according to the present invention are very effective in diagnosing malaria of Korean type where latent period is long, numbers of Protozoa and the antigen in blood are few, and the probability of recurrence is high, because they can diagnose malaria in a latent period. Further, the immunoassay and diagnostic reagent for malaria detecting malaria-specific IgM in blood according to the present invention are useful in an early diagnosis of the malaria patient.

According to the preparation method of the present invention, the Merozoite

Surface Protein of malarial Protozoa with high purity can be prepared more easily and rapidly than prior art techniques by expressing MSP of malarial Protozoa in an active form from a yeast transformant using recombinant DNA technology. The surface protein purified by the preparation method of the present invention has high
5 sensitivity and specificity to antibody as well as high purity. Also, the surface protein purified by the preparation method of the present invention has markedly low pseudo-positive signals, and can be used in a diagnostic reagent and a vaccine for malaria.

10 Further, according to the preparation method of the present invention, the Merozoite Surface Protein of malarial Protozoa with high purity can be prepared more easily and rapidly than prior art techniques by expressing MSP of malarial Protozoa from E. Coli transformant as well as be produced in a large amount.

The surface protein purified by the preparation method of the present
15 invention has high sensitivity and specificity to antibody as well as high purity. Also, the surface protein purified by the preparation method of the present invention has markedly low pseudo-positive signals, and can be used in a diagnostic reagent for malaria.

20 The invention will be further illustrated by the following examples. It will be apparent to those skilled in the art that these examples are given only to illustrate the present invention in more detail, but the invention is not limited to the examples

given.

Example 1-1. Preparation of cDNA from malaria-positive blood

TRI reagent TM (Sigma Co.) was used to purify RNA from malaria-positive
5 blood. The purification method is the following method provided by Sigma Co.
A mixture of 0.1 ml of TRI reagent TM and 0.1 ml of malaria-positive blood was
left for 5 minutes at ambient temperature. 20 μ l of BCP was mixed with the
mixture, and the resulting mixture was left for 5 minutes at ambient temperature and
then was centrifuged for 15 minutes at 12,000 rpm and 4°C. As a result of
10 centrifugation, three layers were formed. Only the upper layer in which RNAs
were contained was transferred to a new tube, and then 50 μ l of isopropanol was
added to the tube. The tube was left for 5 minutes at ambient temperature. The
resulting mixture was centrifuged for 10 minutes at 12,000 rpm and 4°C. The
supernatant was discarded. The pellet was washed with 75% ethanol and
15 dissolved in 20 μ l of deionized distilled water, which was used to prepare cDNA.

9 μ l of the above-purified RNA and 1 μ l of N6 random primer (Genotech)
were mixed and reacted for 5 minutes at 65°C and then transferred to ice for cooling.
4 μ l of RT buffer, 2 μ l of 0.1M DTT(DL-Dithiothreitol)(Sigma, Cal No. D5545), 1
 μ l of 10mM dNTP, 1 μ l of Reverse Transcriptase(Gibco Co.), 1 μ l of RNase
20 inhibitor(Promega) and 1 μ l of deionized distilled water were added to the above
mixture, and the resulting mixture was reacted for 1 hour at 42°C and then reacted

for 10 minutes at 70°C to prepare cDNA.

Example 1-2. preparation of recombinant plasmids comprising genes encoding PV200C.

5 Polymerase chain reaction was performed using cDNAs prepared in Example 1-1 as a template and a pair of primers, SEQ. ID. NO:2 and SEQ. ID. NO:4, as follows.

The reaction mixture containing 5 µl of PCR buffer, 5 µl of 2.5 mM dNTP, 1 µl of sense primer, 1 µl of anti-sense primer, 2 µl of template of cDNA and 35 µl
10 of deionized distilled water was reacted for 30 seconds at 94°C. Thereafter, 1 µl of Vent polymerase(BioLab) was added to the mixture. Then, the reaction having the following cycle was repeated 36 times:

Denaturation 94°C, 30 sec

Primer annealing 55°C, 30 sec

15 Extension 72°C, 30 sec

Again, polymerase chain reaction was performed using the above-amplified DNAs as a template and a pair of primers, SEQ. ID. NO:3 and SEQ. ID. NO:4. The PCR was performed in the same condition as the above condition. The amplified DNAs were confirmed through electrophoresis in 1% agarose gel. The
20 amplified DNAs(referred as “Pv200-ct657” hereafter) and pBluscript KS(+)(Stratagene) vector were cleavaged with restriction enzyme, EcoR V, and

Pv200-ct657 was inserted into pBluscript KS(+) vector by using T4 DNA ligase(Promega). As a result, recombinant plasmids(referred as “pBC-Pv200-ct657” hereafter) were produced. Then, E. coli strain JM 109 was transformed with pBC-Pv200-ct657.

5

Example 1-3. preparation of expression vector pYLJ-MSP

Polymerase chain reaction was performed using pBC-Pv200-ct657 prepared in Example 1-2 as a template and a pair of primers, SEQ. ID. NO:5 and SEQ. ID. NO:6. The PCR was performed in the same condition as the above reaction
10 condition. The amplified DNAs(referred as “Pv200-19” hereafter) were confirmed through electrophoresis in 1% agarose gel.

In order to obtain α -factor leader peptide sequence of yeast to be linked with N-terminus of PV200C polypeptide, PCR was performed using the α -IFN pYLBC(deposit No. KCTC 0051BP) comprising the sequence encoding α -factor
15 leader peptide of yeast as a template and a pair of primers, SEQ. ID. NO:7 and SEQ. ID. NO:8. The PCR was performed in the same condition as the above reaction condition. The amplified DNAs(referred as “ α -leader of yeast” hereafter) were confirmed through electrophoresis in 1% agarose gel.

20 To link the above-amplified Pv200-19 with α -factor leader peptide sequence of yeast, an overlapping PCR was performed using the above two PCR products as templates and a pair of primers, SEQ. ID. NO:6 and SEQ. ID. NO:7. A reaction

mixture containing 5 μ l of PCR buffer, 5 μ l of 2.5 mM dNTP, 1 μ l of sense primer, 1 μ l of anti-sense primer, 1 μ l of PCR products of Pv200-19, 1 μ l of PCR products of α -factor leader peptide of yeast and 35 μ l of deionized distilled water, was reacted for 30 seconds at 94°C. Thereafter, 1 μ l of Vent polymerase(BioLab) was added to the mixture. Then, the reaction having the following cycle was repeated 36 times:

Denaturation 94°C, 30 sec

Primer annealing 55°C, 30 sec

Extension 72°C, 30 sec

10 The amplified DNAs(referred as “ α -Pv200-19” hereafter) were confirmed through electrophoresis in 1% agarose gel.

The above-amplified α -Pv200-19 and pYES-2 vectors were cleavaged with restriction enzymes, HindIII and Xho I, respectively. Then, α -Pv200-19 and pYES-2 vector were linked by T4 ligase to be a recombinant expression vector that is referred as “pYLJ-MSP”(Refer to Figure 1). As a result of analyzing α -Pv200-19 sequence in pYLJ-MSP, it was confirmed that α -Pv200-19 consisted of the genes encoding 108 amino acids as shown SEQ. ID. NO:1. Yeast *Saccharomyces cerevisiae* INVSC1 was transformed with pYLJ-MSP according to Hinnen method(*Proc. Natl. Acad. Sci.*, U.S.A 75, 1929-1933, 1978), and then the yeast transformants were cultivated overnight in minimal medium lacking uracil. The yeast transformants were deposited in Korean Collection for Type Cultures of

Korea Research Institute of Bioscience and Biotechnology deposited on December 18, 1999 (Deposit No. KCTC 0937BP)

Example 1-4. expression and purification of MSP from yeast

5 In order to produce the surface protein MSP from yeast, the transformants pYLJ-MSP/*S. cerevisiae* INVSC1 were inoculated in 100 ml of YEPD medium containing 2% glucose and cultivated overnight. Then, the culture medium was transferred to 2L of YEP medium containing 1% glucose and 1% galactose, respectively, and cultivated for 72 hours at 30°C. The yeast transformants
10 expressed PV200C polypeptide, exhausting glucose. The expressed PV200C polypeptide was secreted into out of cell by the α -factor leader peptide linked with N-terminus of PV200C polypeptide. Thereafter, the α -factor leader peptide was removed by peptidase existing in cell membrane. Accordingly, only the PV200C polypeptide was secreted into culture medium.

15 In order to obtain the PV200C polypeptide secreted into culture medium, the biomass was removed by centrifuging the culture medium that was cultivated for 2 days. Then, only the fluid of culture medium was taken and concentrated by ultrafiltrating apparatus(Amicon, U.S.A.). For easily purifying the concentrated fluid, the surface proteins linked with cationic histidine residues were adsorbed on
20 Probond column(Invitrogen) to which anionic nickels were coupled by using six histidine residues linked with C-terminus of the above-amplified Pv200-19.

The contaminants which were not adsorbed on column were removed by

phosphate buffer containing 10mM imidazol, and then the surface proteins adsorbed on column were desorbed and separated by 500 mM imidazol and phosphate buffer. As shown in Figure 2, the surface proteins having approximately 90% or more of purity were separated by the process. Then, the surface proteins having approximately 99% or more of high purity were obtained by concentrating the separated solution and performing Sephacryl S-200 gel filtration chromatography with the concentrate.

**Example 1-5. assay of antigenicity of the surface protein of
10 malarial Protozoa purified from yeast**

The surface protein PV200C polypeptide obtained in Example 1-4 was analyzed with 12% SDS-PAGE. As a result, it was detected in 18 kDa position, and it was identified as the object protein of the present invention by sequencing amino acids of N-terminus thereof.

15 In addition, Western Blotting was performed with the serum of the malaria patient to assay antigenicity of the surface protein of malarial Protozoa purified from yeast. Western Blotting was performed according to the method suggested by Towbin et al.(*Proc. Natl. Acad. Sci.*, USA, 76, 4350-4354, 1979). After the electrophoresed gel was immersed in a buffer solution containing 25 mM Tris
20 buffer, 190 mM glycine(pH 8.3) and 20% methanol for 30 minutes, the protein was transferred to nitrocellulose membrane by electrophoresis using the same buffer. Then, the protein-bound nitrocellulose membrane was incubated for 1 hour at

ambient temperature in a phosphate buffer(pH 7.4) containing 3% Skim milk. The serum of the malaria patient diluted to 1/100 was added to the membrane and was incubated for 1 hour at ambient temperature. Thereafter, the nitrocellulose membrane was washed 5 times at 5 minute-intervals with 0.05% Tween
5 20/phosphate buffer solution, and then the second antibody(Vector Labs) to which HRP was conjugated was diluted to 1/2000, added to the membrane and incubated for 1 hour at ambient temperature. After the incubation, the nitrocellulose membrane was washed with the above solution, and then both 4-chloro-naphthol and hydrogen peroxide were added to the membrane to induce color development.
10 As a result, it was confirmed that only the PV200C polypeptide was detected at 18 kDa position. Therefore, it was confirmed that the surface protein PV200C polypeptide expressed and purified by the preparation method of the present invention was an antigen having high specificity(refer to Figure 3).

15 **Example 2-1. Preparation of cDNA from malaria-positive blood.**

TRI reagent TM(Sigma Co.) was used to purify RNA from malaria-positive blood. The purification method is the following method provided by Sigma Co. A mixture of 0.1 ml of TRI reagent TM(Sigma Co., Cat. No. T9424) and 0.1 ml of malaria-positive blood was left for 5 minutes at ambient temperature. 20 μ l of
20 BCP was mixed with the mixture, and the resulting mixture was left for 5 minutes at ambient temperature and then was centrifuged for 15 minutes at 12,000 rpm and 4°C. As a result of centrifugation, three layers were formed. Only the top layer

in which RNAs were contained was transferred to a new tube, and then 50 μ l of isopropanol was added to the tube. The tube was left for 5 minutes at ambient temperature. The resulting mixture was centrifuged for 10 minutes at 12,000 rpm and 4°C. The supernatant was discarded. The pellet was washed with 75% ethanol and dissolved in 20 μ l of deionized distilled water treated with DEPC(Diethyl pyrocarbonate), which was used to prepare cDNA.

9 μ l of the above-purified RNA and 1 μ l of N6 random primer (Genotech) were mixed and reacted for 5 minutes at 65°C and then transferred to ice. 4 μ l of PCR buffer, 2 μ l of 0.1M DTT(DL-Dithiothreitol)(Sigma, Cal No. D5545), 1 μ l of 10mM dNTP, 1 μ l of Reverse Transcriptase(Gibco Co.), 1 μ l of RNase inhibitor(Promega) and 1 μ l of deionized distilled water were added to the above mixture, and the resulting mixture was reacted for 1 hour at 42°C and then reacted for 10 minutes at 70°C to prepare cDNA.

15 **Example 2-2. preparation of recombinant plasmids comprising genes encoding PV200C**

Polymerase chain reaction was performed using cDNAs prepared in Example 2-1 as a template and a pair of primers, sense primer and anti-sense primer, having SEQ. ID. NO:9 and SEQ. ID. NO:10, respectively.

20 The reaction mixture containing 5 μ l of PCR buffer, 5 μ l of 2.5 mM dNTP, 1 μ l of sense primer, 1 μ l of anti-sense primer, 2 μ l of template of cDNA and 35 μ l of

deionized distilled water was reacted for 30 seconds at 94°C. Thereafter, 1 µl of Vent polymerase(BioLab) was added to the mixture. Then, the reaction having the following cycle was repeated 36 times:

- | | | |
|---|------------------|---------------|
| | Denaturation | 94°C, 30 sec |
| 5 | Primer annealing | 55°C, 30 sec |
| | Extension | 72°C, 30 sec. |

Again, polymerase chain reaction was performed using the above-amplified DNAs as a template and a pair of primers, sense primer and anti-sense primer, having SEQ. ID. NO:10 and SEQ. ID. NO:11, respectively. The PCR was performed in the same condition as the above condition. It was confirmed that the amplified DNA had a size of approximately 657 bp through electrophoresis in 1% agarose gel.

The amplified DNAs(referred as "Pv200-ct657" hereafter) and pBluscript KS(+)(Stratagene) vector were cleavaged with restriction enzyme, EcoR V, and Pv200-ct657 was inserted into pBluscript KS(+) vector by using T4 DNA ligase(Promega). As a result, recombinant plasmids(referred as "pBC-Pv200-ct657" hereafter) were produced. Then, E. coli strain JM 109 was transformed with pBC-Pv200-ct657 for storage.

20 **Example 2-3. Preparation of expression vector pELK-MSP**

Polymerase chain reaction was performed using pBC-Pv200-ct657 prepared

in Example 2-2 as a template and a pair of primers, sense primer and anti-sense primer, having SEQ. ID. NO:12 and SEQ. ID. NO:13, respectively. It was confirmed that the amplified DNA fragment had a size of approximately 330 bp through electrophoresis in 1% agarose gel.

5 The amplified DNA fragments were purified with phenol/chloroform mixture to produce DNA fragments with high purity. The DNA fragment and vector, pET-19b (Novagen) were cleaved with NdeI and BamHI. Then, the DNA fragment was inserted into pET-19b to prepare an expression vector, pELK-MSP having a size of approximately 6.0 kb (Refer to Figure 4).

10 *E. coli* strain BL21(DE3)lysS was transformed with pELK-MSPs by the treatment with CaCl₂. The transformants were deposited in Korean Collection for Type Cultures of Korea Research Institute of Bioscience and Biotechnology deposited on December 18, 1999 (Deposit No. KCTC 0936BP)

15 **Example 2-4. mass production and purification of PV200C polypeptide in *E. coli***

The transformants pELK-MSP/strain BL21 (KCTC 0936BP) were cultivated for 12 hours in LB medium containing 50 µg/ml of chloramphenicol and 100 µg/ml of ampiciline. Then, 50 ml of the resulting cultures were again inoculated in 1L
20 of LB medium and were cultivated for about 2 hours at 37°C. When absorbance(OD 600) of the cultures became 0.3, IPTG (Isopropyl thio-β-D-galactoside) was added to the medium to a final concentration of 0.2 mM. Then,

the cultures were cultivated further for 7 hours.

After the cultivation, only the cells were isolated by centrifugation. The obtained cells were suspended in 30 ml of Phosphate Buffered Saline(PBS). Then, E. Coli in the suspension was crushed with Sonicator(Branson, Sonifier 450) and
5 centrifuged at 5000 rpm. Because a part of expressed MSP proteins was in the supernatant and the remainder was in the pellet, both the supernatant and the pellet were completely suspended in 4M urea buffer(pH 7.5) where 4M urea was dissolved in PBS, and then were centrifuged, by which only the supernatant was collected.

10 Because the overexpressed surface protein has His marker consisting of 10 histidines in N-terminus thereof, the protein was adsorbed on histidine affinity column(Invitrogen) for purification. The contaminants which were not adsorbed on column were removed repeatedly with a washing buffer in which 10mM imidazol and 0.1M NaCl were dissolved in PBS. Then, the surface proteins
15 adsorbed on column were desorbed and separated with an elution buffer in which 1mM imidazol and 0.1M NaCl were dissolved in PBS. As shown in Figure 5, it was confirmed by 15% SDS PAGE that the surface proteins having approximately 90% or more of purity were separated by the process.

Then, it was confirmed that PV200C polypeptide having approximately 99%
20 or more of high purity were obtained from recombinant E.Coli by concentrating the separated solution and performing Sephacryl S-200 (Pharmacia Co., Sweden) gel filtration chromatography with the concentrate.

Example 2-5. assay of antigenicity of the surface protein of malarial Protozoa purified from E. coli

Western Blotting(Towbin et al, *Proc. Natl. Acad. Sci.* 76, 4350-4354, 1979)
5 was performed with the serum of the malaria patient to assay antigenicity of the MSP PV200C prepared in Example 2-4.

The PV200C prepared in Example 2-4 was loaded into 15% SDS-PAGE gel with a concentration of 5 μ g per well and electrophoresed. After the electrophoresed gel was immersed in a transfer buffer solution containing 25mM
10 Tris-HCl, 190 mM glycine (pH 8.3) and 20% methanol for 30 minutes, the protein was transferred to nitrocellulose membrane(Hafer Transphor Power Lid, Pharmacia Co.). Then, the protein-bound nitrocellulose membrane was blocked for 1 hour at ambient temperature in a blocking buffer(3% Skim milk /PBS, pH 7.4). The serum of the malaria patient diluted to 1/100 was added to the membrane and was
15 incubated for 1 hour at ambient temperature.

Thereafter, the nitrocellulose membrane was washed 5 times at 5 minute-intervals with a washing buffer solution(0.05% Tween/PBS). The second antibody (Vector Labs, U.S.A.) to which HRP was conjugated was diluted to 1/2000, added to the membrane and incubated for 1 hour at ambient temperature. After the
20 incubation, the nitrocellulose membrane was washed with the washing buffer solution, and then both 4-chloro-naphthol and hydrogen peroxide were added to the membrane to induce color development.

As a result, it was confirmed that only the surface protein PV200C polypeptide was blotted at 15 kDa position. Therefore, it was confirmed that the surface protein PV200C polypeptide expressed and purified by the preparation method of the present invention was an antigen having high specificity. (Refer to 5 Figure 6)

Example 3-1. Preparation of well plate coated with the surface antigens of malarial Protozoa

To provide a solid support to be used for the diagnosis of malaria, the 10 solution of recombinant surface antigens of malarial Protozoa prepared in Example 1-4 or 2-4 was diluted with 0.1M carbonate buffer (pH 9.5) to 0.5 µg/ml, and 100 µl of the solution was added to each well of 96-well plate. To adsorb the surface antigens on the well, the well plate was sealed tightly and left for 18 hours at 4 °C. Then, the surface antigens which were not adsorbed on the well were removed by 15 adding 300 µl of phosphate buffer solution containing 5% general goat serum to each well and leaving it for 18 hours at 4 °C. After the solution that remained in the well was discarded, moisture was removed by leaving the well plate for 1 hour at ambient temperature. Then, the well plate was transferred to a hermetic container with a dehumidifying agent and stored in a refrigerator at 4 °C.

20 In the embodiment of the present invention, the above-prepared well plate coated with the surface antigens of malarial Protozoa was used as a solid support in

a diagnostic reagent and immunoassay for malaria.

Example 3-2. Diagnosis of malaria by indirect enzyme immunoassay

5 To diagnose malaria by indirect enzyme immunoassay, the solid support prepared by coating 96-well plate(Nunc) with recombinant surface antigens prepared in Example 1-4 or 2-4 according to the method of Example 3-1, and the labeled antigen conjugate consisting of HRP and anti-human IgM antibody or anti-human IgG antibody derived from goat were used.

10 100 μ l of a sample dilution solution containing 1.15 mg/ml NaOH, 0.2 mg/ml of KH_2PO_4 , 0.2 mg/ml KCl, 8 mg/ml NaCl, 300 μ l/ml bovine serum, 0.2 mg/ml thimerosal and the like was added to each well of 96-well plate(Nunc) coated with recombinant surface antigens according to the method of Example 3-1. Then, 10 μ l of antibody-negative plasma sample or 10 μ l of
15 antibody-positive plasma sample was added to the wells, and the resulting mixtures were well mixed. The well plate was incubated for 60 minutes in a reactor at 37 °C. After the reaction terminated, the well plate was washed 5 times with 300 μ l of a phosphate buffer solution containing 0.05% Tween 20. Anti-human IgG antibody(Cappel) or anti-human IgM antibody(Bethyl)
20 derived from goat to which HRP was conjugated was diluted to 1/40,000 with a dilution solution containing 10mM Tris, 0.5M NaCl, 1mM CaCl_2 , 0.5% glycerol, 0.2 ml/ml bovine serum, 100 μ l/ml goat serum, 0.05% Tween 20, 0.2

mg/ml thimerosal and 50 µg/ml phenol red. 100 µl of the diluted conjugate was added to each well of the 96-well plate and the well plate was incubated for 60 minutes at 37 °C. After the reaction terminated, the well plate was washed 5 times with 0.05% Tween 20/phosphate buffer solution. 100 µl of a substrate solution containing 100 µg/ml tetramethylbenzidine, 0.006% hydrogen peroxide and citrate-phosphate buffer solution(pH 4.5) was added to each well. After color development in a dark place for 30 minutes, 100 µl of the reaction-stopping solution (2N sulfuric acid solution) was added to each well to terminate the color development reaction. Then, the absorbance was measured at 450 nm (reference wavelength, 650 nm) by using the 96 well plate reader (Molecular Devices, USA). Tetramethylbenzidine that is a color fixing agent in the substrate solution was decomposed by HRP conjugated to anti-human IgG antibody or anti-human IgM antibody, to induce the color development. Then, existence of malaria-specific antibodies and amount of the antibodies were detected by measuring the absorbance.

According to the above method, 20 malaria-positive samples(plasma of the malaria patients) and 48 malaria-negative samples(plasma of normal people) were diagnosed for malaria. When positive cut-off value was determined by adding 0.5 to the mean of the negative samples, according to the indirect enzyme immunoassay using the recombinant surface antigens of malarial Protozoa according to the present invention, 18 out of the 20 malaria patients' samples were judged to be positive and 46 out of 48 normal people's samples were judged to be negative.

Therefore, the indirect enzyme immunoassay of the present invention exhibited 90% of sensitivity and 95.8% of specificity.

Example 3-3. Preparation of the surface antigen of malarial

5 Protozoa conjugated to HRP

The labeled antigen conjugates to be used for antigen sandwich enzyme immunoassay were prepared.

The recombinant surface antigens of malarial Protozoa prepared in Example 1-4 or 2-4 was dialyzed for 1 day at 4 °C against 1 L of 0.01 M sodium carbonate
10 buffer solution(pH 9.6). The buffer solution was exchanged 3 times during the dialysis.

Also, 5 mg of HRP was dissolved in 0.5 ml of distilled water in a tube, and then 100 µl of 42 mg/ml NaIO₄ was added to the HRP solution. After the tube was wrapped with foil, it was shaken for 30 minutes at ambient temperature for the
15 oxidation reaction. After HRP was oxidized enough, 60 µl of 1M glycerol was added to the reaction solution. Thereafter, the tube wrapped with foil was shaken for 30 minutes at ambient temperature to terminate the oxidation reaction.

For a conjugation reaction of HRP and the surface antigen, salts were removed from the HRP reaction solution by PD10 column (Pharmacia) saturated
20 with 0.01 M sodium carbonate buffer (pH 9.6). The above solution of the surface antigens was added to the oxidized HRP reaction solution. Then, the tube was wrapped with foil and shaken overnight at ambient temperature to prepare

conjugates of HRPs and the surface antigens. After the conjugation reaction of HRP and the surface antigen was terminated, in order to stabilize the HRP-surface antigen conjugates, 40.8 μ l of 4 mg/ml NaBH₄ solution was added to the tube, and the tube wrapped with foil was shaken at 4 °C for 2 hours for the reaction.

5 To remove HRPs in the reaction solution which were not conjugated, the surface proteins conjugated to HRPs were adsorbed on Probond column that was equilibrated with phosphate buffer solution by utilizing histidine residues linked with C-terminus of the surface protein. Then, HRPs which were not conjugated were removed by phosphate buffer solution. The HRP-surface antigen conjugates
10 adsorbed on Probond column were desorbed by phosphate buffer solution containing 1M imidazole. The conjugates were collected. The protein concentration of the collected HRP-surface antigen conjugates was determined by using commercially available BSA protein quantitative test kit. The sample was stored in a refrigerator after addition of bovine serum albumin (BSA) of final
15 concentration of 1 % .

The above-prepared HRP-surface antigen conjugates were used as labeled antigen conjugates in an antigen sandwich enzyme immunoassay and diagnostic reagent for malaria.

20 **Example 3-4. Diagnosis of malaria by antigen sandwich enzyme immunoassay using HRP-surface antigen conjugates**

Antigen sandwich enzyme immunoassay for malaria was performed by using

the HRP-surface antigen conjugates prepared in Example 3-3.

30 μ l of the solution of the HRP-surface antigen conjugates was added to each well of 96-well plate(Nunc) coated with recombinant surface antigens according to the method of Example 3-1. Then, 100 μ l of malaria-positive samples(plasmas of malaria patients) or 100 μ l of malaria-negative samples(plasmas of normal people) was added to the wells, and the resulting mixtures were well mixed. The well plate was incubated for 90 minutes in a reactor at 37 °C. After the reaction terminated, the well plate was washed 5 times with each 300 μ l of a phosphate buffer solution containing Tween 20. 100 μ l of a substrate solution containing 100 μ g/ml tetramethylbenzidine, 0.006% hydrogen peroxide and citrate-phosphate buffer solution(pH 4.5) was added to each well of the well plate. After color development of the plate in a dark place for 30 minutes, 100 μ l of the reaction-stopping solution (2N sulfuric acid solution) was added to each well to terminate the color development reaction. Then, the absorbance was measured at 450 nm (reference wavelength, 650 nm) by using the 96 well plate reader (Molecular Devices, USA). Tetramethylbenzidine that is a color fixing agent in the substrate solution was decomposed by HRP conjugated to the surface antigen, to induce the color development. Then, existence of malaria-specific antibodies and amount of the antibodies were detected by measuring the absorbance.

According to the above method, 202 malaria-positive samples(plasmas of malaria patients) and 400 malaria-negative samples(plasmas of normal people) were diagnosed for malaria. When positive cut-off value was determined by adding 0.5

to the mean of the negative samples, according to the antigen sandwich enzyme immunoassay using the HRP-surface antigen conjugates according to the present invention, 199 out of 202_malaria patients' samples were judged to be positive and 398 out of the 400_normal people's samples were judged to be negative. Therefore, 5 the antigen sandwich enzyme immunoassay of the present invention exhibited 98.5% of sensitivity and 99.6% of specificity.

Example 4-1. Diagnosis of malaria by IgM capture enzyme immunoassay

10 To diagnose malaria by IgM capture enzyme immunoassay, a solid support coated with anti-human IgM antibodies derived from goat, and the labeled antigen conjugates consisting of HRP and recombinant surface antigens purified from yeast or E.Coli transformant were used.

The plate was prepared as follows: anti-human IgM antibodies (anti- 15 human IgM mu chain-specific, affinity purified, Bethyl Co., USA) derived from goat were diluted with phosphate buffer solution (pH 7.0) to 1~10 µg/ml and then 100~200 µl of the mixture was added to each well of the polystyrene plate. The well plate was left for approximately 12~18 hours at ambient temperature, and the solution that remained in the well was removed by 20 suction. 250 µl of 0.1% gelatin or 0.1% casein dissolved in phosphate buffer solution was added to each well, and the well plate was left for 2 hours at ambient temperature. Then, the solution that remained in the well was

removed by suction. The well plate was dried for 18 hours in a cold room and used.

100 μ l of a sample dilution solution that is a phosphate buffer solution containing 0.1% bovine serum albumin was added to each well of the well
5 plate coated with anti-human IgM antibodies derived from goat. Then, 2~20 μ l of antibody-negative plasma sample or 2~20 μ l of antibody-positive plasma sample was added to the wells, and the resulting mixtures were well mixed. The well plate was incubated for 60 minutes in a reactor at 37 °C. After the reaction terminated, the well plate was washed 5 times with 300 μ l of a
10 phosphate buffer solution containing 0.05% Tween 20.

The surface antigens of malarial Protozoa purified from yeast or E.Coli transformant which were prepared in Example 1-4 or 2-4 were conjugated with HRPs according to the method of Example 3-3 and then used.

The above-prepared conjugates consisting of HRPs and the surface
15 antigens were diluted to 1:100 to 1:1,000 with phosphate buffer solution (pH 7.0) containing 1% BSA and 0.05% Tween 20. Then, 100 μ l of the solution of the HRP-surface antigen conjugates was added to each well of the well plate. The well plate was incubated for 30 minutes at 37 °C. After the reaction terminated, the well plate was washed 5 times with phosphate buffer
20 solution containing 0.05% Tween 20. 100 μ l of a substrate solution containing 100 μ g/ml tetramethylbenzidine, 0.006% hydrogen peroxide and citrate-phosphate buffer solution(pH 4.5) was added to each well of the plate.

After color development in a dark place for 30 minutes, 100 μ l of the reaction-stopping solution (1N sulfuric acid solution) was added to each well to terminate the color development reaction. Then, the absorbance was measured at 450 nm (reference wavelength, 650 nm) by using the 96 well
5 plate reader (Molecular Devices, USA). Tetramethylbenzidine that is a color fixing agent in the substrate solution was decomposed by HRP of the labeled antigen conjugate, to induce the color development. Then, existence of malaria-specific IgM and amount of the IgM were detected by measuring the absorbance.

10 According to the above method, 216 malaria-positive samples(plasmas of malaria patients) and 353 malaria-negative samples(plasmas of normal people) were diagnosed for malaria. When positive cut-off value was determined by adding 0.05 to the mean of the negative samples, according to IgM capture enzyme immunoassay using the
15 recombinant surface antigen of malarial Protozoa according to the present invention, 212 out of 216 malaria patients' samples were judged to be positive and 351 of 353 normal people's samples were judged to be negative. Therefore, the IgM capture enzyme immunoassay of the present invention exhibited 98.1% of sensitivity and 99.4% of specificity (Refer to Figure 7).

20

Example 4-2. Comparison of IgM capture enzyme immunoassay with indirect enzyme immunoassay

In the IgM capture enzyme immunoassay, a solid support coated with anti-human IgM antibodies derived from goat, and the labeled antigen conjugates consisting of HRPs and the recombinant surface antigens purified from yeast or E.Coli transformant were used. The IgM capture enzyme immunoassay was
5 performed according to the method of Example 4-1.

Also, the indirect enzyme immunoassay was performed according to the method of Example 3-2.

To compare the IgM capture enzyme immunoassay with the indirect enzyme immunoassay, 75 malaria-positive samples(plasmas of malaria
10 patients) and 92 malaria-negative samples(plasmas of normal people) were diagnosed for malaria. When positive cut-off value was determined by adding 0.05 to the mean of the negative samples, according to the IgM capture enzyme immunoassay using the recombinant surface antigens of malarial
Protozoa according to the present invention, 73 out of 75 malaria patients'
15 samples were judged to be positive and 92 of 92 normal people's samples were judged to be negative. Therefore, the IgM capture enzyme immunoassay of the present invention exhibited 97.3% of sensitivity and
100% of specificity.

In the meantime, when positive cut-off value was determined by adding 0.2
20 to the mean of the negative samples, according to the indirect enzyme immunoassay of the present invention, 59 out of 75 malaria patients' samples were judged to be positive and 85 of 92 normal people's samples were judged to be negative.

Therefore, the indirect enzyme immunoassay of the present invention exhibited 78.6% of sensitivity and 92.4% of specificity.(Refer to Figure 8).

**Example 4-3. Comparison of IgM capture enzyme immunoassay
5 with antigen sandwich enzyme immunoassay in normal people classified
by age**

In the IgM capture enzyme immunoassay, a solid support coated with anti-human IgM antibodies derived from goat, and the labeled antigen conjugates consisting of HRPs and the recombinant surface antigens purified from yeast or
10 E.Coli transformant were used. The IgM capture enzyme immunoassay was performed according to the method of Example 4-1.

Also, the antigen sandwich enzyme immunoassay was performed according to the method of Example 3-4.

To compare the IgM capture enzyme immunoassay with the antigen
15 sandwich enzyme immunoassay, plasma samples of 129 normal people were diagnosed for malaria. The 129 normal people consisted of 26 people in teen, 43 people in twenties, 33 in thirties and 27 in forties.

When positive cut-off value was determined by adding 0.05 to the mean of the negative samples, according to the IgM capture enzyme
20 immunoassay using the recombinant surface antigens of malarial Protozoa according to the present invention, 129 of 129 normal people's samples were judged to be negative. Therefore, the IgM capture enzyme immunoassay of

the present invention exhibited 100% of specificity.

In the meantime, when positive cut-off value was determined by adding 0.05 to the mean of the negative samples, according to the antigen sandwich enzyme immunoassay of the present invention, 127 of 129 normal
5 people's samples were judged to be negative. Therefore, the antigen sandwich enzyme immunoassay of the present invention exhibited 98.4% of specificity. Especially, when plasma samples of normal people in thirties or less who were born after the prevalence period of malaria were diagnosed for malaria, the antigen sandwich enzyme immunoassay of the present invention
10 exhibited 100% of specificity. However, when plasma samples of normal people in forties who were born in the prevalence period of malaria were diagnosed for malaria, 2 of 27 normal people's samples were judged to be positive and the antigen sandwich enzyme immunoassay of the present invention exhibited 92.6% of specificity. Therefore, the specificity of the
15 antigen sandwich enzyme immunoassay of the present invention decreased in specific ages (Refer to Figure 9).

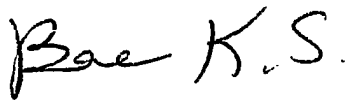
BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT
OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1

TO : SUNG, Jae-Kap
 LG Chemical LTD.
 #104-1, Moonji-dong, Yusong-ku, Taejon 305-380,,
 Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21/pELK-MSP	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCTC 0936BP
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: <input checked="" type="checkbox"/> a scientific description <input type="checkbox"/> a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on December 18 1999 and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on January 10 2001	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Korean Collection for Type Cultures Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of authorized official(s):  BAE, Kyung Sook, Director Date: January 12 2001

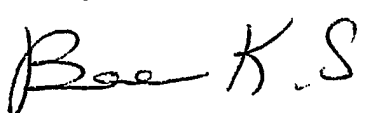
BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT
OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

INTERNATIONAL FORM

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TO : SUNG, Jae-Kap
 LG Chemical LTD.
 #104-1, Moonji-dong, Yusong-ku, Taejon 305-380,,
 Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Saccharomyces cerevisiae</i> INVScI/pYLJ-MSP	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCTC 0937BP
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: <input checked="" type="checkbox"/> a scientific description <input type="checkbox"/> a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on December 18 1999 and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on January 10 2001	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Korean Collection for Type Cultures Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of authorized official(s):  BAE, Kyung Sook, Director Date: January 12 2001

Claims

1. An immunoassay for malaria detecting malaria-specific antibodies in blood by using antigens of malarial Protozoa.
- 5 2. The immunoassay for malaria according to Claim 1, wherein said detected malaria-specific antibodies are malaria-specific IgM.
3. The immunoassay for malaria according to Claim 2, wherein said immunoassay detecting malaria-specific IgM in blood is an IgM capture immunoassay.
- 10 4. The immunoassay for malaria according to Claim 3, wherein said IgM capture immunoassay is an IgM capture enzyme immunoassay.
5. The immunoassay for malaria according to Claim 2, wherein said malaria-
15 specific IgM in blood is detected by using anti-human IgM antibody.
6. The immunoassay for malaria according to Claim 1, wherein said immunoassay detecting malaria-specific antibodies in blood is an enzyme immunoassay.
- 20 7. The immunoassay for malaria according to Claim 1 or 2, wherein said antigen of malarial Protozoa is a surface antigen of malarial Protozoa.

8. The immunoassay for malaria according to Claim 7, wherein said surface antigen of malarial Protozoa is a part or whole of Merozoite Surface Protein of *Plasmodium Vivax*.
- 5 9. The immunoassay for malaria according to Claim 8, wherein said part or whole of Merozoite Surface Protein of *Plasmodium Vivax* is C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*.
- 10 10. The immunoassay for malaria according to Claim 9, wherein said C-terminus of Merozoite Surface Protein of *Plasmodium Vivax* is a part or whole of PV200C polypeptide in the C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*.
11. The immunoassay for malaria according to Claim 10, wherein amino acid sequences of the PV200C polypeptide are amino acid sequences as shown in SEQ.
15 ID NO : 1.
12. The immunoassay for malaria according to Claim 1 or 2, wherein said antigen of malarial Protozoa is a recombinant antigen.
- 20 13. An immunoassay for malaria detecting malaria-specific antibodies in blood, comprising :
- (a) immobilizing surface antigens of malarial Protozoa on a solid support;

- (b) pouring blood serum or plasma sample to combine malaria-specific antibodies with the antigens immobilized on the solid support;
- (c) adding labeled antigen conjugates consisting of antigens and markers to combine the labeled antigen conjugates with the malaria-specific antibodies;
- 5 and
- (d) analyzing by using the markers of the labeled antigen conjugates bound to the malaria-specific antibodies.

14. The immunoassay for malaria according to Claim 13, comprising :
- 10 (a') expressing Merozoite surface antigens of *Plasmodium Vivax* from transformants and purifying it;
 - (a) immobilizing the purified surface antigens on a solid support;
 - (b) pouring blood serum or plasma sample to combine malaria-specific antibodies with the surface antigens immobilized on the solid support;
 - 15 (c) adding labeled antigen conjugates consisting of antigens and markers to combine the labeled antigen conjugates with the malaria-specific antibodies;
 - (d) inducing color development by using the markers of the labeled antigen conjugates bound to the malaria-specific antibodies; and
 - (e) measuring the absorbance by using a 96-well plate reader.

20

15. The immunoassay for malaria according to Claim 13, wherein the immunoassay for malaria is an indirect immunoassay, and the antigen of the labeled

antigen conjugate in the step (c) is anti-human IgG antibody and/or anti-human IgM antibody.

16. The immunoassay for malaria according to Claim 13, wherein the
5 immunoassay for malaria is an antigen sandwich immunoassay, and the antigen of the labeled antigen conjugate in the step (c) is a surface antigen of malarial Protozoa.

17. The immunoassay according to Claim 16, wherein the antigen of the labeled antigen conjugate in the step (c) is a part or whole of Merozoite Surface Protein of
10 *Plasmodium Vivax*.

18. An IgM capture immunoassay for malaria detecting malaria-specific IgM in blood, comprising :

- (i) immobilizing anti-human IgM antibodies on a solid support;
- 15 (ii) pouring blood serum or plasma sample to combine malaria-specific IgM with the anti-human IgM antibodies immobilized on the solid support;
- (iii) adding labeled antigen conjugates consisting of markers and antigens of malarial Protozoa to combine the labeled antigen conjugates with the malaria-specific IgM; and
- 20 (iv) analyzing by using the markers of the labeled antigen conjugates bound to the malaria-specific IgM.

19. The immunoassay for malaria according to Claim 18, wherein the antigen of the labeled antigen conjugate in the step (iii) is a surface antigen of malarial Protozoa.
- 5 20. The immunoassay according to Claim 19, wherein the antigen of the labeled antigen conjugate in the step (iii) is a part or whole of Merozoite Surface Protein of *Plasmodium Vivax*.
21. A diagnostic reagent for malaria detecting malaria-specific antibodies in blood,
10 comprising antigens of malarial Protozoa.
22. The diagnostic reagent for malaria according to Claim 21, wherein said detected malaria-specific antibodies are malaria-specific IgM.
- 15 23. The diagnostic reagent for malaria according to Claim 21 or 22, wherein said antigen of malarial Protozoa is a surface antigen of malarial Protozoa.
24. The diagnostic reagent for malaria according to Claim 23, wherein said surface antigen of malarial Protozoa is a part or whole of Merozoite Surface Protein
20 of *Plasmodium Vivax*.
25. The diagnostic reagent for malaria according to Claim 24, wherein said part or

whole of Merozoite Surface Protein of *Plasmodium Vivax* is C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*.

26. The diagnostic reagent for malaria according to Claim 25, wherein said C-terminus of Merozoite Surface Protein of *Plasmodium Vivax* is a part or whole of PV200C polypeptide in the C-terminus of Merozoite Surface Protein of *Plasmodium Vivax*.

27. The diagnostic reagent for malaria according to Claim 26, wherein amino acid sequences of the PV200C polypeptide are amino acid sequences as shown in SEQ. ID NO : 1.

28. The diagnostic reagent for malaria according to Claim 21 or 22, wherein said antigen of malarial Protozoa is a recombinant antigen.

15

29. A diagnostic reagent for malaria detecting malaria-specific antibodies in blood, comprising :

a solid support coated with surface antigens of malarial Protozoa;

labeled antigen conjugates consisting of antigens and markers; and

20 a substrate solution containing a color fixing agent.

30. The diagnostic reagent for malaria according to Claim 29, wherein said

surface antigen of malarial Protozoa is a part or whole of Merozoite Surface Protein of *Plasmodium Vivax*.

31. The diagnostic reagent for malaria according to Claim 29, wherein the marker
5 of the labeled antigen conjugate is horse radish peroxidase, alkaline phosphatase, colloidal gold, fluorescent materials, dyes or the like.

32. The diagnostic reagent for malaria according to Claim 29, wherein the
substrate solution consists of a buffer solution and a color fixing agent which
10 induces color development when reacted with the marker of the labeled antigen conjugate.

33. The diagnostic reagent for malaria according to Claim 29, wherein the antigen
of the labeled antigen conjugate is anti-human IgG antibody and/or anti-human IgM
15 antibody.

34. The diagnostic reagent for malaria according to Claim 29, wherein the antigen
of the labeled antigen conjugate is a surface antigen of malarial Protozoa.

20 35. The diagnostic reagent for malaria according to Claim 34, wherein the antigen of the labeled antigen conjugate is a part or whole of Merozoite Surface Protein of *Plasmodium Vivax*.

36. A diagnostic reagent for malaria detecting malaria-specific IgM in blood, comprising :

a solid support coated with anti-human IgM antibodies;

5 labeled antigen conjugates consisting of markers and antigens of malarial Protozoa; and

a substrate solution containing a color fixing agent.

37. The diagnostic reagent for malaria according to Claim 36, wherein the antigen
10 of the labeled antigen conjugate is a surface antigen of malarial Protozoa.

38. The diagnostic reagent for malaria according to Claim 37, wherein the antigen of the labeled antigen conjugate is a part or whole of Merozoite Surface Protein of *Plasmodium Vivax*.

15

39. The diagnostic reagent for malaria according to Claim 36, wherein the marker of the labeled antigen conjugate is horse radish peroxidase, alkaline phosphatase, colloidal gold, fluorescent materials, dyes or the like.

20 40. The diagnostic reagent for malaria according to Claim 36, wherein the substrate solution consists of a buffer solution and a color fixing agent which induces color development when reacted with the marker of the labeled antigen

conjugate.

41. An expression vector comprising genes of Merozoite Surface Protein of *Plasmodium Vivax*, α -factor leader peptide of yeast and histidine residues.

5

42. The expression vector according to Claim 41, wherein the genes of Merozoite Surface Protein comprise a part or whole of genes of Merozoite Surface Protein of *Plasmodium Vivax*.

10 43. The expression vector according to Claim 42, wherein the genes of Merozoite Surface Protein are genes of PV200C polypeptide in the C-terminus of Merozoite Surface Protein.

44. The expression vector according to Claim 43, wherein amino acid sequences
15 of the PV200C polypeptide are amino acid sequences as shown in SEQ. ID NO : 1.

45. The expression vector according to Claim 44, wherein the expression vector is pYLJ-MSP having a cloning map as shown in Fig.1.

20 46. A yeast transformant pYLJ-MSP/*S. cerevisiae* INVSC1 (Deposit No. KCTC 0937BP) transformed with the expression vector according to claim 45.

47. A method for preparing Merozoite Surface Protein of malarial Protozoa by using a yeast transformant.
48. The method according to Claim 47, comprising
- 5 (i) preparing an expression vector of Merozoite Surface Protein of malarial Protozoa;
- (ii) transforming the expression vector into a yeast to obtain a yeast transformant;
- (iii) culturing the yeast transformant to obtain surface proteins; and
- 10 (iv) separating and purifying the surface proteins.
49. The method according to Claim 48, wherein the step (iv) uses a column having an affinity for histidine and gel filtration chromatography.
- 15 50. A diagnostic reagent for malaria, comprising the surface proteins produced by the preparation method according to Claim 47.
51. An expression vector comprising genes of Merozoite Surface Protein of *Plasmodium Vivax* , histidine marker and T7 promotor.
- 20 52. The expression vector according to Claim 51, wherein the genes of Merozoite Surface Protein comprise a part or whole of genes of Merozoite Surface Protein of

Plasmodium Vivax.

53. The expression vector according to Claim 52, wherein the genes of Merozoite Surface Protein are genes of PV200C polypeptide in the C-terminus of Merozoite
- 5 Surface Protein.
54. The expression vector according to Claim 53, wherein amino acid sequences of the PV200C polypeptide are amino acid sequences as shown in SEQ. ID NO : 1.
- 10 55. The expression vector according to Claim 54, wherein the expression vector is pELK-MSP having a cloning map as shown in Fig. 4.
56. E. Coli transformant pELK-MSP/BL21 (Deposit No. KCTC 0936BP) transformed with the expression vector according to claim 55.
- 15
57. A method for preparing Merozoite Surface Protein of malarial Protozoa by using E. Coli transformant.
58. The method according to Claim 57, comprising
- 20 (i) preparing an expression vector of Merozoite Surface Protein of malarial Protozoa;
- (ii) transforming the expression vector into E. Coli to obtain E. Coli

transformant;

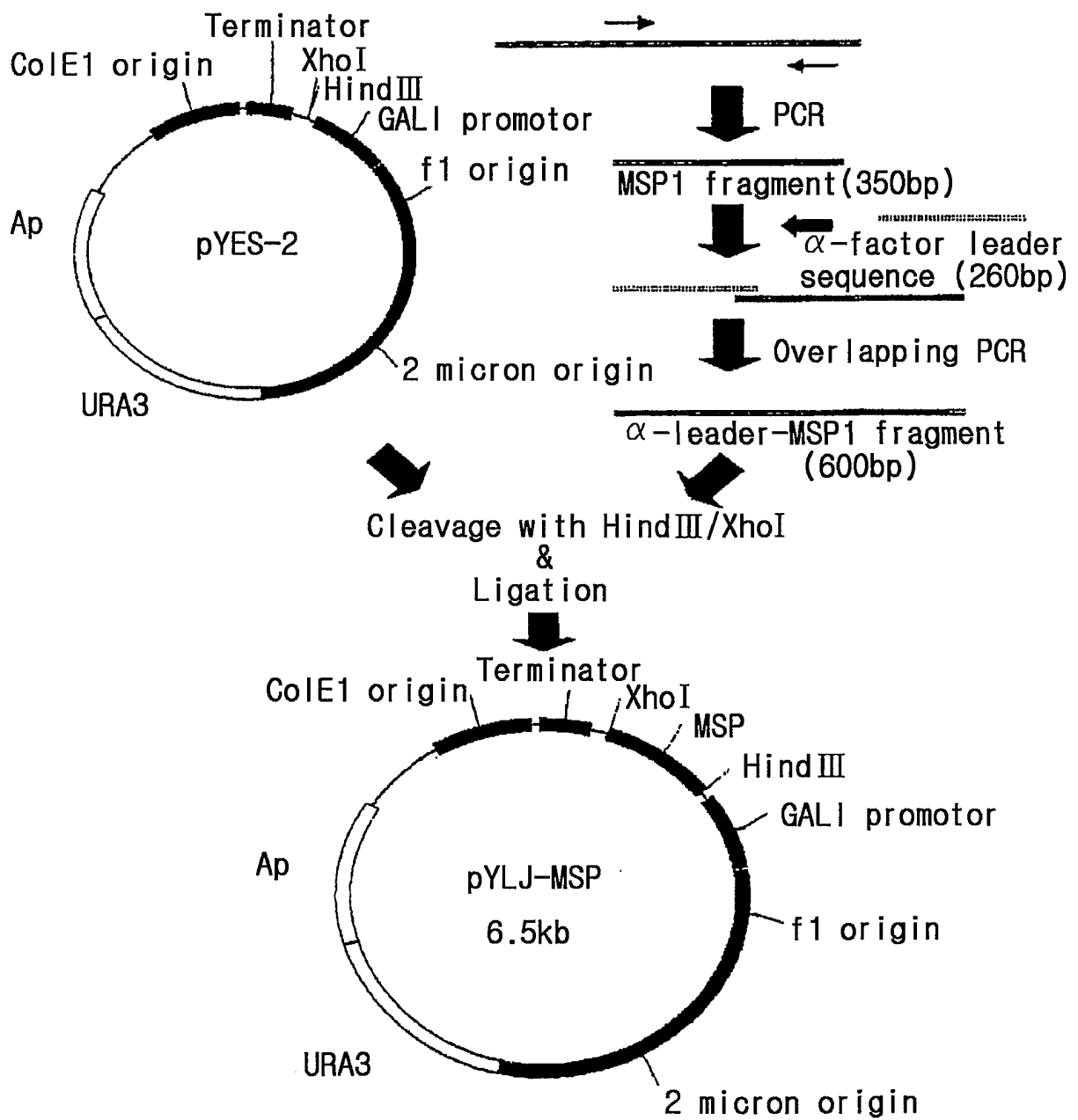
(iii) culturing the E. Coli transformant to obtain surface proteins; and

(iv) separating and purifying the surface proteins.

5 59. The method according to Claim 58, wherein the step (iv) uses a column having an affinity for histidine and gel filtration chromatography.

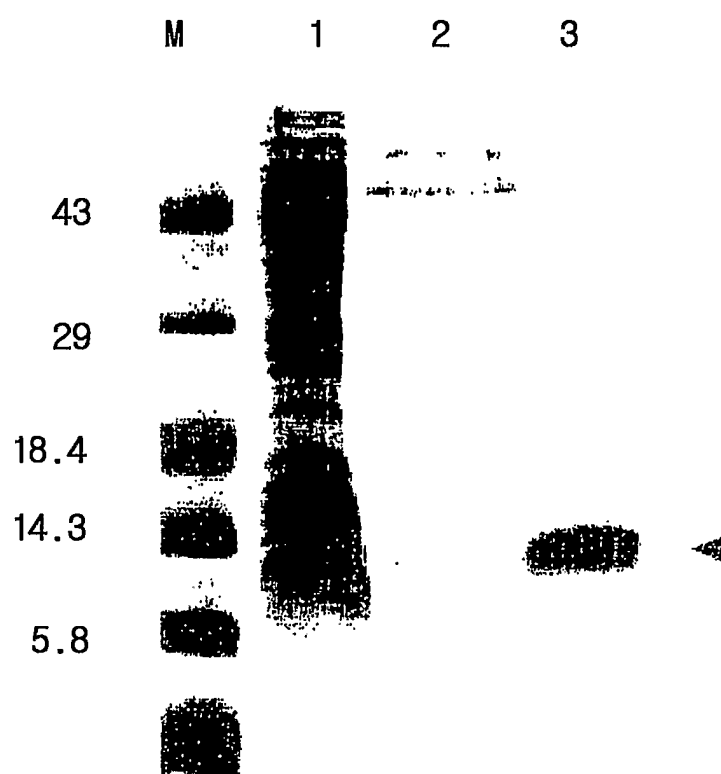
60. A diagnostic reagent for malaria, comprising the surface proteins produced by the preparation method according to Claim 57.

Fig. 1



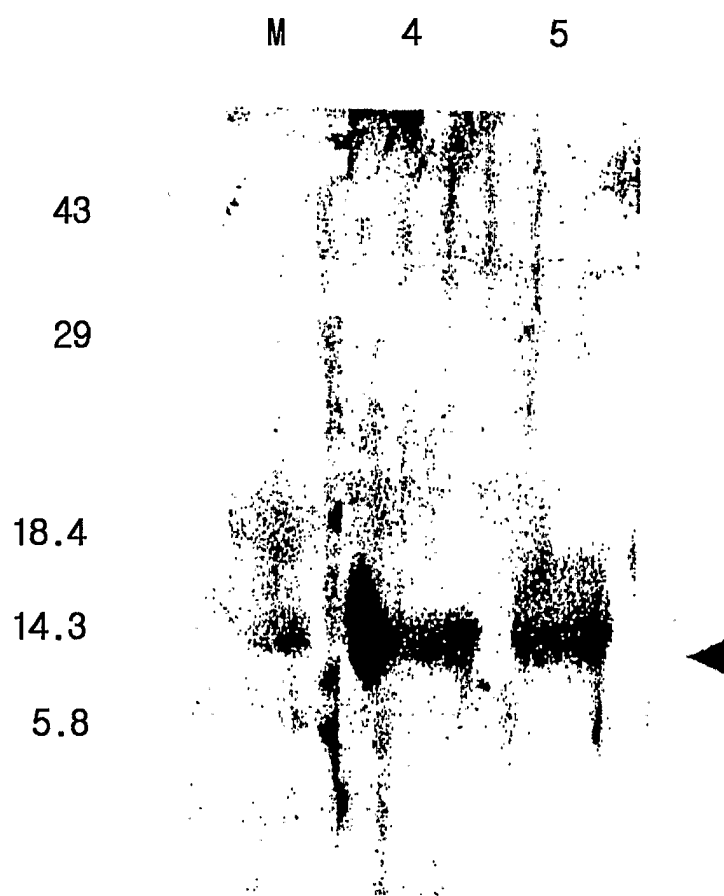
2/9

Fig. 2



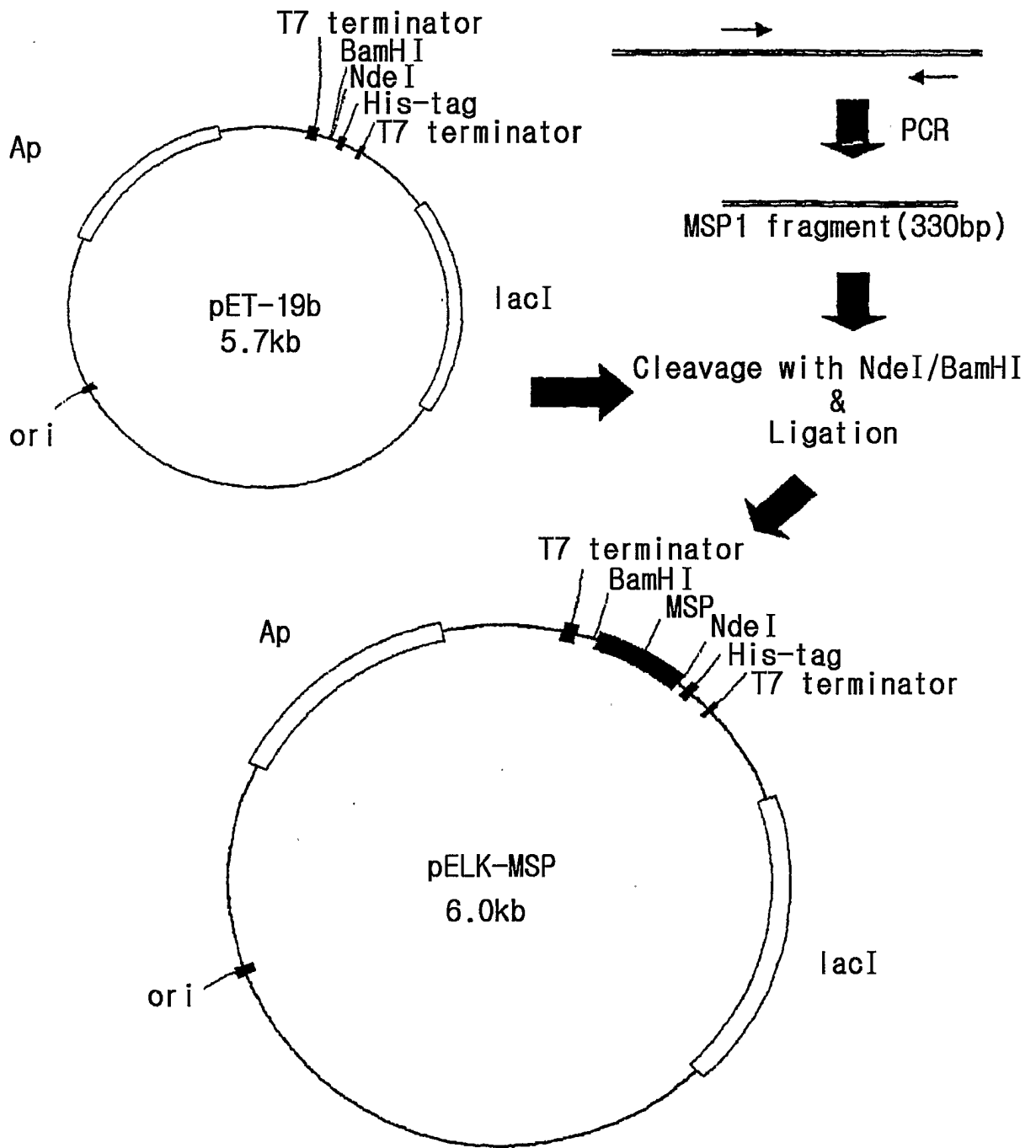
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Fig. 3



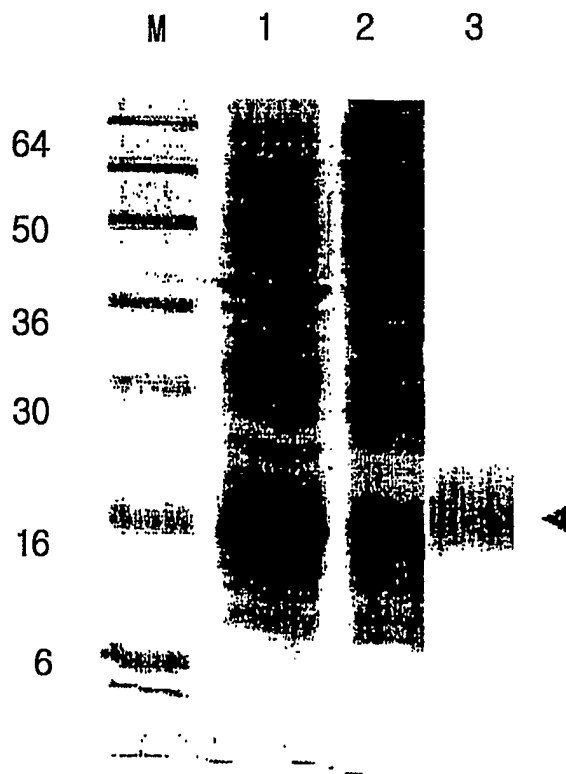
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Fig. 4



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Fig. 5



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Fig. 6

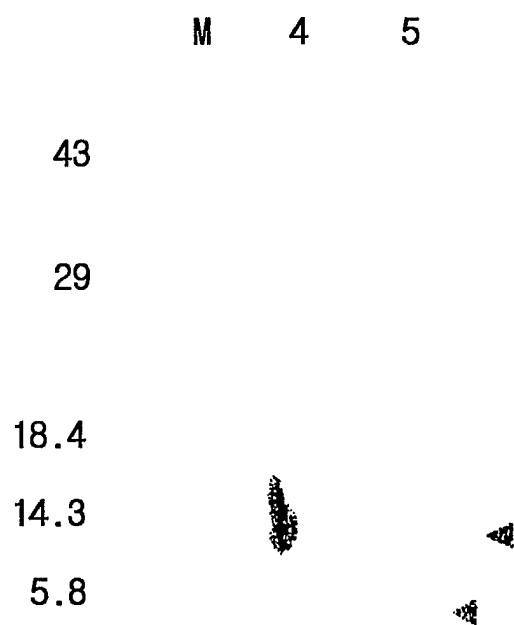


Fig. 7

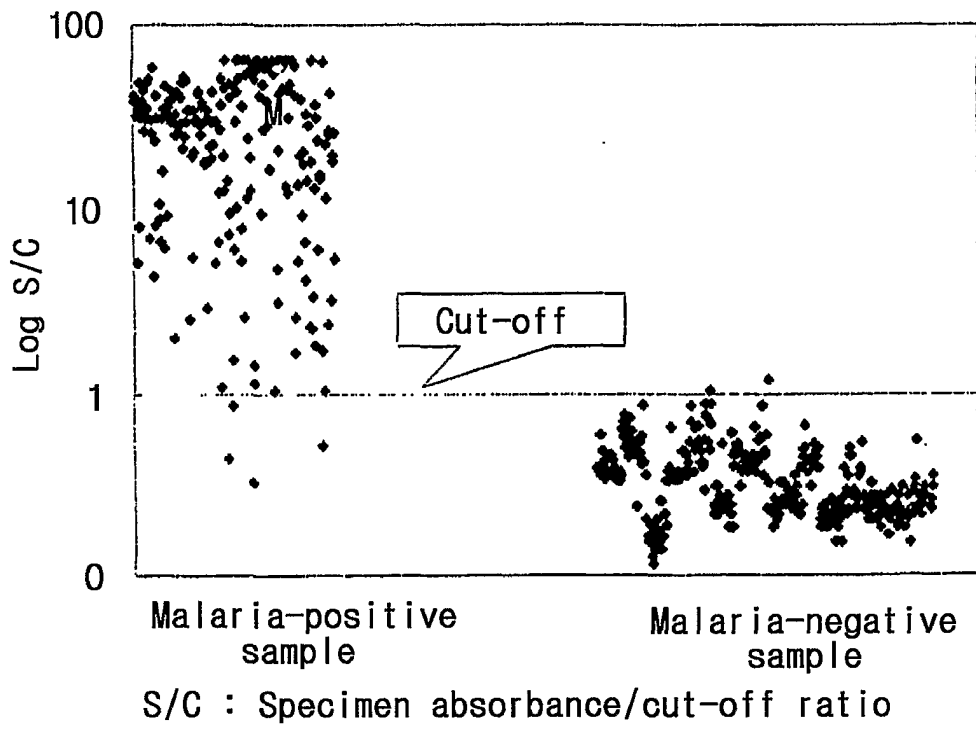


Fig. 8

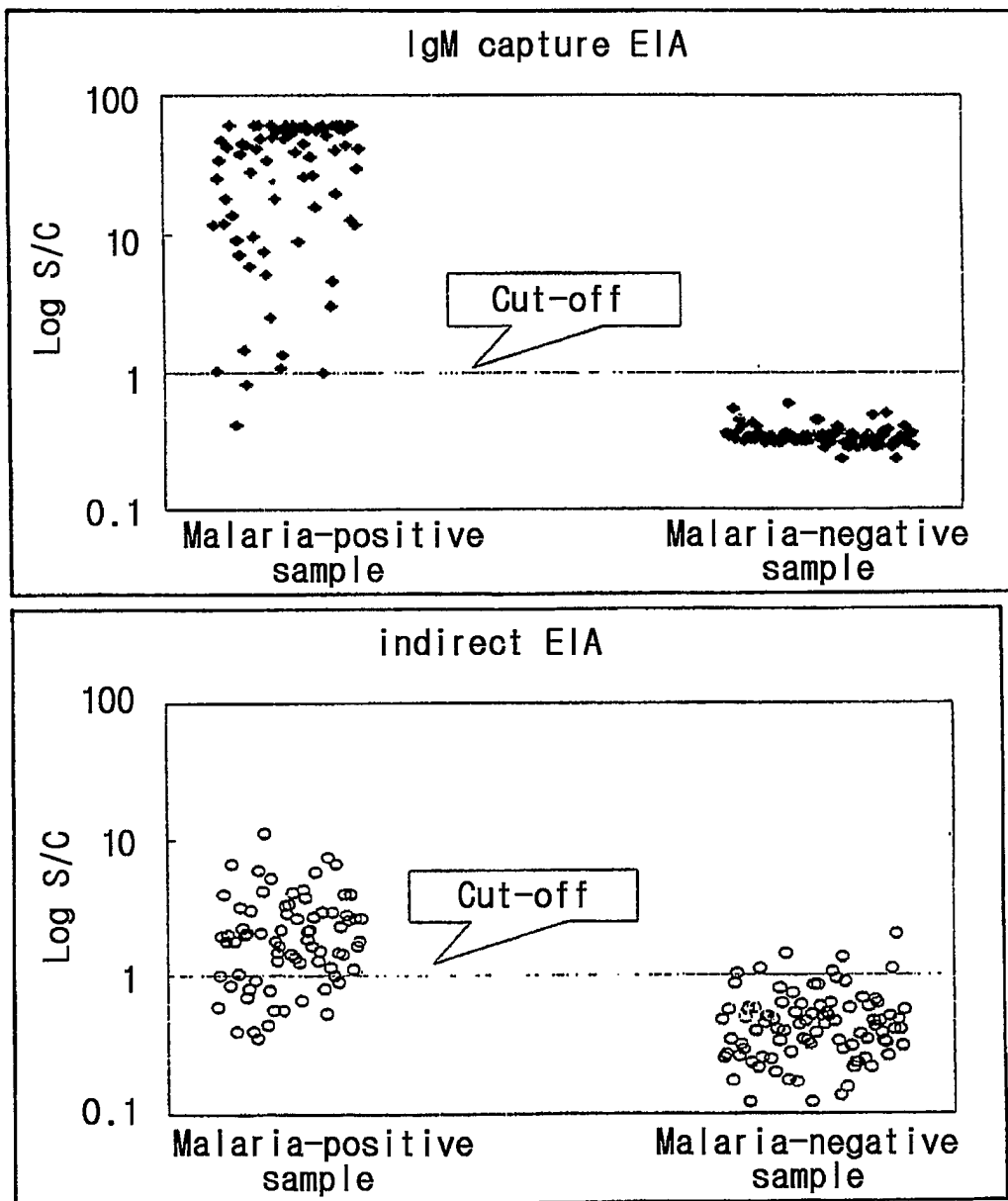
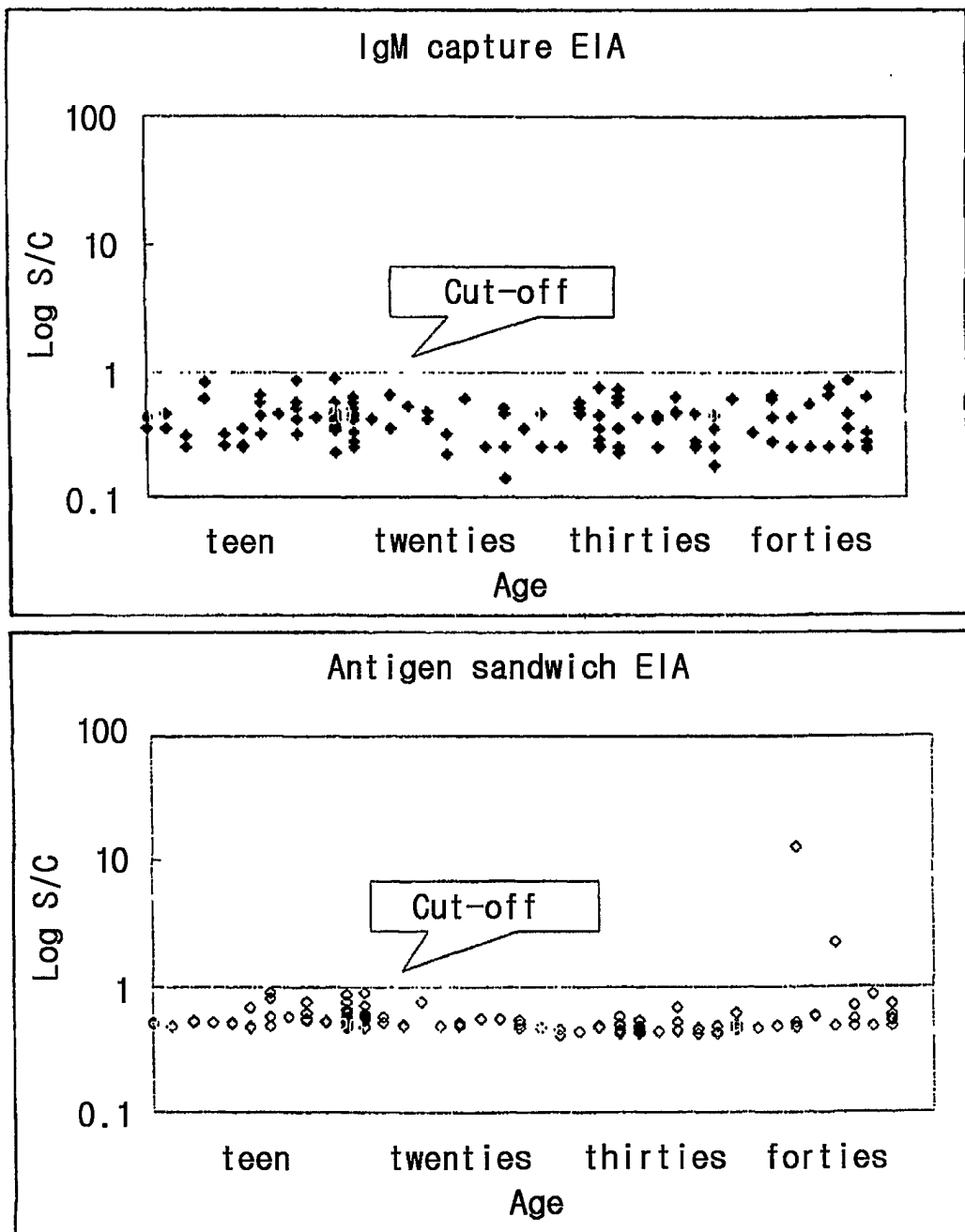


Fig. 9



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Asp Asn Ala Ala Cys Tyr Arg Tyr Leu Asp Gly Thr Glu Glu Trp Arg
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Cys Leu Leu Thr Phe Lys Glu Glu Gly Gly Lys Cys Val Pro Ala Ser
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Asn Val Thr Cys Lys Asp Asn Asn Gly Gly Cys Ala Pro Glu Ala Glu
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40

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR01/00229

A. CLASSIFICATION OF SUBJECT MATTER		
IPC7 C12Q 1/04, G01N 33/53, C12N 15/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) IPC7 C12Q 1/04, G01N 33/53, C12N 15/00		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Korean Patent applications for inventions since 1975		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Medline "malaria and plamodium and vivax"		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97/26911 A1 (GEORGE TOWN UNIVERSITY) 31 JULY 1997	1,6,7,21-35
Y	SOARES et al. 'Acquired immune responses to the N- and C-terminal regions of Plasmodium vivax merozoite surface protein 1 in individuals exposed to malaria' In: Infect Immun, 1997, Vol. 65, 1606-1614	1,6-17, 21-35
Y	PAN et al. 'Vaccine candidate MSP-1 from Plasmodium falciparum: a redesigned 4917 bp polynucleotide enables synthesis and isolation of full-length protein from Escherichia coli and mammalian cells.' In: Nucleic Acids Res, 1999, 02. 15., Vol.27, 1094-1103	1,6-12, 21-35
Y	Fruh et al. 'A new tool for the serodiagnosis of acute Plasmodium falciparum malaria in individuals with primary infection' In J Immunol Methods', 1989, 08. 15., Vol.122, 25-32	1, 6-17, 21-35
Y	Sanchez et al. 'Antibody response to Plasmodium vivax antigens in human malaria' In: Am J Trop Med Hyg, 1994, 03., Vol.50, 329-338	1, 6-17, 21-35
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<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 25 JUNE 2001 (25.06.2001)		Date of mailing of the international search report 25 JUNE 2001 (25.06.2001)
Name and mailing address of the ISA/KR Korean Intellectual Property Office Government Complex-Daejeon, Dunsan-dong, Seo-gu, Daejeon Metropolitan City 302-701, Republic of Korea Facsimile No. 82-42-472-7140		Authorized officer HAN, Hyun Sook Telephone No. 82-42-481-5596



INTERNATIONAL SEARCH REPORT

international application No.

PCT/KR01/00229

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	Maiern et al. 'Serodiagnosis of malaria. Plasmodium berghei and P. falciparum as antigen for the indirect immune of fluorescence test' In:Immun Infekt, 1997.06. Vol.7, 75-82	21-35, 50, 60
Y	Kaslow et al. 'Expression and immunogenicity of the C-terminus of a major blood-stage surface protein of Plasmodium vivax, Pv200(19), secreted from Saccharomyces cerevisiae' In:Immunol Lett, 1996. 07. Vol.51, 187-189	41-55
Y	Kaslow et al. 'Expression and antigenicity of Plasmodium falciparum major merozoite surface protein (MSP1(19)) variants secreted from Saccharomyces cerevisiae' In:Mol. Biochem Parasitol, 1994, Vol.63, 283-289	41-55
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Y	Witzgall et al. 'A mammalian expression vector for the expression of GAL4 fusion proteins with an epitope tag and histidine tail.' In:Anal Biochem, 1994, Vol.223, 291-298	41-47, 51-60
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Y	HUW DAVIES Introductory Immunology, Chapman & Hall, 1997, pages 381-383	13-17
T	Park et al. 'Naturally acquired antibody responses to the C-terminal region of merozoite surface protein 1 of Plasmodium vivax in Korea' , In: Clin Diagn Lab Immunol, 2001, Vol.8, 14-20	1-60

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR01/00229

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9726911 A1	31.07.97	AU 2249097 JP 2000504223	20.08.97 11.04.00
WO 9730158	21.08.97	EP 880588 CA 2241802	02-12-98 21-08-97

专利名称(译)	免疫测定和疟疾诊断试剂		
公开(公告)号	EP1255852A1	公开(公告)日	2002-11-13
申请号	EP2001908398	申请日	2001-02-15
[标]申请(专利权)人(译)	LG化学投资		
申请(专利权)人(译)	LG化学投资有限公司		
当前申请(专利权)人(译)	LG化学投资有限公司		
[标]发明人	LIM KOOK JIN SHON MI JIN YOO SEUNG BUM LEE SANG IK OH JAE HOON LEE SEUNG WON KIM HYUNG CHEOL		
发明人	LIM, KOOK-JIN SHON, MI-JIN YOO, SEUNG-BUM LEE, SANG-IK OH, JAE-HOON LEE, SEUNG-WON KIM, HYUNG-CHEOL		
IPC分类号	C07K14/445 G01N33/569 C12Q1/04 G01N33/53 C12N15/00		
CPC分类号	C07K14/445 G01N33/56905 G01N2333/445 Y02A50/58		
优先权	1020000007648 2000-02-17 KR 1020000007649 2000-02-17 KR 1020000007650 2000-02-17 KR 102000012172 2000-03-10 KR 1020000045806 2000-08-08 KR		
其他公开文献	EP1255852B9 EP1255852A4 EP1255852B1		
外部链接	Espacenet		

摘要(译)

本发明涉及通过使用疟疾原生动物的抗原进行疟疾的免疫测定和诊断试剂。更优选地，本发明涉及用于疟疾的免疫测定和诊断试剂，其通过使用间日疟原虫的裂殖子表面蛋白来检测血液中的疟疾特异性抗体。根据本发明的检测血液中疟疾特异性抗体的免疫测定和诊断试剂具有高特异性和灵敏度，并且可用于诊断潜伏期长的疟疾类型和血液中原生动物的数量。此外，本发明涉及使用酵母或大肠杆菌制备疟疾原生动物的表面蛋白的方法。优选地，本发明提供了表达载体，其包含间日疟原虫的裂殖子表面蛋白和组氨酸残基的基因，以及用表达载体转化的转化体。另外，本发明提供了一种利用转化体制备疟原虫的裂殖子表面蛋白的方法。由根据本发明的酵母或大肠杆菌转化体制备的疟疾原生动物的表面蛋白裂殖子表面蛋白对抗体具有高灵敏度和特异性以及高纯度。而且，通过本发明的制备方法制备的表面蛋白具有显著低的假阳性信号，并且可用于诊断疟疾。

