Malaria Screen ELISA
Catalog No. KAPRMLS37

DIAsource ImmunoAssays SA	Phone: + 32 67 88 99 99
Rue de l'Industrie 8	Fax: + 32 67 88 99 90
1400 Nivelles	Email : tech.support@diasource.be
Belgium	Website : www.diasource.be

INTENDED USE

The DIAsource KAPRMLS37 Malaria Screen ELISA is intended for use for the detection of antibodies to *P.falciparum, P.vivax, P.ovale* and *P.malariae* in human serum.

SUMMARY AND EXPLANATION

Malaria is one of the most common diseases in the world. More than half the world population lives in malaria-infected areas. Over 200 million cases annually result in up to 3 million deaths each year; a majority of which are in young children. In non-endemic areas, it is one of the most important imported diseases, resulting in a number of deaths in late-diagnosed or unsuspected cases each year.

The disease is caused by protozoa of the genus *Plasmodium*, transmitted by the bite of the female *Anopheles* mosquito. There are four species causing human malaria: *P.falciparum, P.vivax, P. malariae,* and *P. ovale*. The disease may also be transmitted by transfusion of infected blood. Once in the blood the sporozoite makes its way to the liver where for the next 2 weeks merozoites are produced. These are released into the blood where they invade the red cells and produce more merozoites, causing the cells to rupture. It is this rupturing that is responsible for the clinical symptoms.

Of the four species, *P. falciparum* is the most common and the most virulent, causing most malaria-related deaths. *P. vivax* is the next most common cause of malaria. Although rarely fatal, this form of malaria can be accompanied by severe clinical symptoms. It is a common cause of malaria in S.E. Asia and S. America.

People infected with *Plasmodium* spp. form antibodies in response. The DIAsource MALARIA ELISA kit is designed to detect antibodies occurring in subjects infected with *P.falciparum, P.vivax, P.ovale* and *P.malariae*

PRINCIPLE OF THE TEST

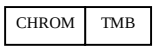
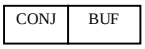
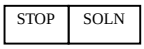
The KAPRMLS37 Malaria Screen kits use four recombinant antigens in a sandwich test to produce a test that is both highly specific and sensitive. The antigens will detect *P.falciparum, P.vivax, P.ovale* and *P.malariae* -specific IgG, IgM, and IgA enabling the test to detect antibodies during all stages of infection. All reagents except the Conjugate and Wash solution are supplied ready to use and colour-coded, and the procedure uses undiluted samples and standard volumes for ease of both manual and automated use. The assay can be used with both serum.

The plastic wells are coated with a mixture of *P. falciparum* and *P. vivax* recombinant antigens. The antigenic similarity between *Plasmodium* species means that antibodies to all species can be detected. Specific antibodies in serum or plasma specimens combine with these antigens and with the same antigens conjugated to horseradish peroxidase, when conjugate is added to a well in which the specimen has been incubated. After unreacted material has been removed by washing, the presence of bound enzyme indicating the presence in the specimen of specific antibodies is revealed by a colour change in the substrate/chromogen mixture. The intensity of the colour is compared to that in control wells to determine the presence or absence of specific antibody.

REAGENTS

The DIAsource *Malaria Screen* ELISA kit contains sufficient reagent for 96 wells. Each kit contains the following reagents:

MATERIAL PROVIDED

Antigen-Coated Microtitration Strip		Quantity : 1 plate
Wash Concentrate		Quantity : 1 bottle
Substrate		Quantity : 1 bottle
Negative Control		Quantity : 1 vial
Positive Control		Quantity : 1 vial
Conjugate		Quantity : 1 bottle
Conjugate Dilution Buffer		Quantity : 1 bottle
Stopping Solution		Quantity : 1 bottle

MATERIAL NOT PROVIDED

Properly calibrated and maintained pipetting devices capable of delivering volumes of 50 microlitres (specimens and reagents) and approx 300 microlitres (wash fluids).
Plate or strip reader to read at 450nm and (optionally) at a wavelength between 620 and 690 nm.
37 degree C incubator .
Malaria ELISA may be automated for both liquid handling and result interpretation. A variety of systems have been used for this – please consult the manufacturers of both the kit and the automation system for advice on automation.
Equipment should be able to support the following tolerances :
Volume dispensed +/- 10%.
Incubation temperature +/- 2 °C .
Incubation time +/- 2 minutes.

Antigen-Coated Microtitration Strips

One stripholder containing 12x8 (96) microtitration wells coated with Malaria recombinant antigen. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package, and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

Wash Concentrate

1x 125 mL, Saline containing surfactant, Dilute Wash Buffer 1 in 20 with distilled or deionised water prior to use.
Store at 2-8°C until expiration date.

Malaria Controls

Two vials, negative (2ml) and positive (1,5ml) of human serum.
The Negative control must be tested three times with each lot of tests, and the Positive control twice.
Store at 2-8°C until expiration date.

Conjugate

One bottle, 0.8 mL. Recombinant antigens conjugated to horseradish peroxidase. Store at 2-8°C until expiration date.
Dilute conjugate 1 + 10 in Conjugate Buffer. (50 µl + 500 µl per 10 wells)

Conjugate Dilution Buffer
One bottle, 8 mL. Buffered saline containing surfactant and stabilisers. Store at 2-8°C until expiration date.

Substrate
One bottle, 7 mL. Urea peroxide and tetramethyl benzidine. Store at 2-8°C until expiration date.

Stopping Solution
One bottle, 7 mL, containing 0.5M H2SO4 in solution. Store at 2-8°C until expiration date.

PRECAUTIONS

For *in vitro* use

The following universal Good Laboratory Practices should be observed:

Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose of all reagents and materials in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit may contain some reagents made with human source material (e.g. serum or plasma) or used in conjunction with human source materials. The material in this kit has been tested by CE marked methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION

Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes, and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.

For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION AND HANDLING

Serum should be used, and the usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at –20°C. Do not use hemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

PREPARATION FOR ASSAY

A thorough understanding of this package insert is necessary for successful use of the product.Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affected the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the Substrate Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate and wash buffer. Avoid contamination of the Substrate Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and Substrate Chromogen Solution can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

PREPARATION OF REAGENTS:

Wash Solution

Dilute Wash Buffer 1 in 20 with distilled or deionised water prior to use.

Microtitration Strips

Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

Conjugate

Dilute conjugate 1 + 10 in Conjugate Buffer. (50 µl + 500 µl per 10 wells)

Assay Procedure

Bring all reagents and specimens to room temperature prior to use.

Assay controls

The Negative control must be tested three times with each lot of tests, and the Positive control twice.

Verification of Specimen addition

Verification is by detecting photometrically the difference between an empty well and a well containing serum or plasma at a wavelength of 450 nm. Wells containing specimen will have an A450 reading of between 0.050 and 1.000.

Procedural notes

Washing must be thorough, with complete filling and emptying of the wells at each cycle

Procedure:

Add **50 µl of the undiluted sample** (or control – see "Assay Controls" above) to a coated well.
Mix on a plate shaker for 30 seconds.
Incubate (covered) at 37o C for 30 minutes.

Wash

5 x with working strength wash buffer.
A short soak time of about 30 seconds is recommended between each wash cycle.
Tap out excess liquid.

Conjugate Incubation
Add **50 µl diluted conjugate** to each well.
Incubate (covered) at 37o C for 30 minutes.
Addition of conjugate is verified by reading at 450/ nm. A well with conjugate added must have an OD greater than 0.2

Wash
5 x with working strength wash buffer.
A short soak time of about 30 seconds is recommended between each wash cycle.
Tap out excess liquid.

Substrate Incubation
Add **50 µl** substrate/chromogen mixture to each well.
Incubate at room temperature for 30 minutes.
There is a clear difference of colour between an empty well and a well containing substrate.
Addition of substrate is verified by reading at 550 nm .
A well with substrate added must have an A550 of greater than 0.080
As the substrate is photosensitive, it is recommended that the plate be protected from light during this incubation.

Stop Colour Development
Add **50 µl** 0.5M sulphuric acid to each well. (Blue colour changes to yellow).

Read Results
Read at 450 nm (A450)
Use of a reference filter at 620 – 690 nm will eliminate effects of scratches, bubbles, etc

Cut-Off Value
Calculated as the mean of the negative control values plus 0.100
i.e. $\frac{\text{Negative Control 1 + NC2 + NC3}}{3} + 0.100$

Example: $\frac{0.030 + 0.025 + 0.035}{3} = 0.030$

Cut-Off Value= 0.030 + 0.100 = 0.130

RESULTS
Calculate the mean absorbance for each control and unknown.
A450 of each Negative Control should be lower or equal to 0.080 If one control is above this value the reading should be ignored and the cut–off calculated using the remaining two.
A450 of each Positive Control should be greater than or equal to 1.000

Interpretation
Samples with an A450 value less than the Cut-off value are considered negative by Malaria Screen ELISA.
Samples just below the Cut-off (C.O. –10% A450) should however, be interpreted with caution. It is advisable to retest the corresponding samples in duplicate when the systems and laboratory procedures permit.
Re-tested samples that are above the cut-off in at least one duplicate are considered positive and should be investigated further. Samples that are below the cut-off in both duplicates are considered to be negative.

Performance Characteristics

Specificity
Clinical study showed that DIAsource Malaria Screen ELISA has a specificity of 96.21%

Sensitivity
External data for 76 acute *P.falciparum* cases showed 92.5% (95% confidence limits 90.5% - 94.5%)
External data for 258 IFAT ≥ 80 for *P.falciparum* showed 94.4% (95% confidence limits 92.44% - 96.38%)
Internal data for *P.vivax showed* 100% (95% confidence limits 97.63% - 100%)
Only small numbers of samples from *P.ovale* and *P.malariae* infections have been studied. Sensitivity for these was 80% and 67% respectively. Numbers were too small to allow meaningful statistical analysis. These figures will be updated as more samples from these infections are tested

Precision

Specimen No.	No. of replicates	Mean A450-A620	Intra-assay CV (%)	Inter-assay CV (%)
1	16	2,402	2,28	3,78
2	16	1,316	3,83	5,17
3	16	0,672	3,83	5,52
4	16	0,353	4,06	6,15
5	16	0,195	3,19	6,16
6 (Negative)	16	0,046	6,95	6,84

REFERENCES

1. Kitchen A.D. et al Evaluation of a malarial antibody assay for use in the screening of blood and tissue products for clinical use . Vox Sanguinis (2004) **87**, 150 - 155
2. Seed C.R. et al The efficacy of a malarial antibody enzyme immunoassay for establishing the reinstatement status of blood donors potentially exposed to malaria Vox Sanguinis (2005) **88**, 98 – 106
3. Kitchen A.D. et al Tranfusion transmitted malaria: current donor selection guidelines are not sufficient. Vox Sanguinis (2005) **88**, 200 – 201

Consult instructions for	Manufacturer
Storage temperature	Contains sufficient for n tests
Use by	In vitro diagnostic medical device
LOT Batch code	REF Catalogue number

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