Malaria Screen ELISA

Catalog No. E-MLS-K37

IVD

2°c 8°c

RD-RatioDiagnostics	Phone: + 49 69 78074942
60489 Frankfurt	Fax: + 49 69 78074998
Westerbachstr.47	Email. info@rd-labs.com
Germany	

CE

INTENDED USE

The RD-RatioDiagnostics E-MLS-K37 Malaria Screen ELISA is intended for use for the detection of antibodies to 3 major spc.of Malaria parasite in human serum and plasma.

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 and malariae*. 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.
People infected with *Plasmodium* spp. form antibodies in response. The RD-RatiDiagnostics MALARIA ELISA kit is designed to detect antibodies occurring in subjects infected with *P.spc*

PRINCIPLE OF THE TEST

The E-MLS-K37 Malaria Screen kits uses 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* and *P.malariae* -specific IgG, IgM, and IgA enabling the test to detect antibodies during all stages of infection. The plastic wells are coated with a mixture of recombinant antigens of four spc. malaria parasite. 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 TMB substrate. The intensity of the colour is compared to that in control wells to determine the presence or absence of specific antibody.

REAGENTS

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

MATERIAL PROVIDED	QUANTITY	CATALOG NO.
Antigen-Coated Microtitration Strip	One Plate	E-MLS-10
Wash Concentrate	One Bottle	E-WSL-30
Substrate	One Bottle	E-TMB-08
Negative control	One Vial	E-MLS-01
Positive control	One Vial	E-MLS-02
Conjugate	One Bottle	E-MLS-20
Stopping Solution	One Bottle	E-STP-10

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 TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB 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 TMB 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.

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 material. The material in this kit has been tested by CE recommended 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.

MATERIAL NOT PROVIDED

MATERIAL NOT PROVIDED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 μL 100 μL, and 1 mL
- Semi-automatic pipette to deliver 100 μL
- Automatic microtitration plate washer
- Absorbent material for blotting the strips
- Incubator

MATERIAL PROVIDED

he RD-RatioDiagnostics *Malaria Screen* ELISA kit contains sufficient reagent for 96 wells. Each kit contains the following reagents:

MATERIAL PROVIDED	QUANTITY	CATALOG NO.
Antigen-Coated Microtitration Strip	One Plate	E-MLS-10
Wash Concentrate	One Bottle	E-WSL-30
Substrate	One Bottle	E-TMB-08
Negative control	One Vial	E-MLS-01
Positive control	One Vial	E-MLS-02
Conjugate	One Bottle	E-MLS-20
Stopping Solution	One Bottle	E-STP-10

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:
One bottle, 100 mL, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% Brij weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

***Malaria Controls*:**
Two vials, negative (1ml) and positive of human serum diluted in buffer.
The Negative control and positive control must be tested two times with each run.
Store at 2-8°C until expiration date.

Conjugate:
One bottle, 6 ml. Ready to use conjugate. Store at 2-8°C until expiration date.

TMB-Substrate:
One bottle, 6 mL, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.

Stopping Solution:
One bottle, 6 ml, containing 0.5M H_2SO_4 in solution. Store at 2-8°C until expiration date.

PREPARATION OF REAGENTS:
Wash Solution:
Dilute Wash Buffer 1 in 10 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.

Assay Procedure:
Bring all reagents and specimens to room temperature prior to use.

Assay controls
The Negative and positive control must be tested two times with each run.

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

Procedure:

Add **50 µl of ready to use negative and positive control in each defined well (duplicate) and add 50 µl of the samples in other wells.**
Incubate at 37° C for 45 minutes.

Wash
Aspirate and wash each well four (6) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 0.35 mL of the Wash Solution into each well, and (c) repeat step (a) and (b) six times.

Conjugate Incubation
Add **50 µl ready to use conjugate** to each well.
at 37° C for 45 minutes.

Wash
Aspirate and wash each well four (6) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 0.35 mL of the Wash Solution into each well, and (c) repeat step (a) and (b) six times.

TMB Substrate
Add **50 µl** TMB-substrate to each well.
Incubate at room temperature for 30 minutes in dark.

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 0.200
i.e. $\frac{\text{Negative Control 1 + Negative Control 2}}{2} + 0.200$

Example: $\frac{0.030 + 0.025}{2} + 0.200$

∴ Cut-Off Value= 0.027 + 0.200 = 0.227

RESULTS
Calculate the mean absorbance for each control and unknown.

OD of 450 of each Negative Control should be lower or equal to 0.120. If one control is above this value, the reading should be ignored and the cut–off calculated using the remaining two.
OD of 450 of each Positive Control should be greater than or equal to 0.260.

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 and Sensitivity
Evaluation study showed that RD Malaria Screen ELISA has a specificity of 100% and Sensitivity 95 %

2. Precision

2. Inter-assay Study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	0.123	0.457	1.036
SD	0.00	0.01	006
CV%	**3.4**	**1.7**	**5.7**

3. Intra-assay study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	0.022	0.88	0.86
SD	0.003	0.067	0.069
CV%	**14.45**	**7.6**	**8.08**