



## User's Manual

# Malaria-Ab ELISA Kit

**REF** DEIABL353

 96T

**RUO**

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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## PRODUCT INFORMATION

### Intended Use

The Malaria-Ab ELISA is intended for the qualitative determination of IgG/IgM antibodies against Plasmodium in human serum or plasma (citrate, heparin).

### General Description

Malaria is the most common tropical parasitic disease and is by now also of importance through returning travelers in non-endemic areas. The transmission of Plasmodium (*P. ovale*, *P. malariae*, *P. vivax*, *P. falciparum*) occurs by the bite of the Anopheles female mosquito. *P. falciparum* and *P. vivax* are the most common types.

The initial symptoms of infection are nonspecific (headache, fever, chills, dizziness, nausea) and may persist for several days. Several days or weeks later it comes to periodic fever attacks every 3 to 4 days depending on the species. For the Tropical Malaria these periodic phases are often not pronounced.

Especially at risk are children and non-immune adults from non-endemic areas (tourists from USA and Europe) as well as children from endemic areas between 6 months and 3 years of age. Adults in endemic regions develop a kind of resistance to malaria.

Alternative routes of infection are transplacentally, through blood transfusion or contaminated syringes among drug addicts.

Infection or presence of pathogen may be identified by:

1. Microscopy ("blood smear", Giemsa stain)
2. PCR
3. Serology: e.g. ELISA

### Principles of Testing

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.

The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

### Reagents And Materials Provided

1. **Microtiter Plate:** 12 break-apart 8-well snap-off strips coated with recombinant Plasmodium antigens (P.

falciparum, P. vivax); in resealable aluminium foil.

2. **Conjugate:** 1 bottle containing 20 ml of peroxidase labelled antibodies to human IgG/IgM in phosphate buffer (10 mM); colored blue; ready to use; black cap.
3. **Positive Control:** 1 vial containing 2 ml control; colored yellow; ready to use; red cap;  $\leq 0.02\%$  (v/v) MIT.
4. **Negative Control:** 1 vial containing 2 ml control; colored yellow; ready to use; blue cap;  $\leq 0.0015\%$  (v/v) CMIT/ MIT (3:1).
5. **Cut-off Control:** 1 vial containing 3 ml control; colored yellow; ready to use; green cap;  $\leq 0.02\%$  (v/v) MIT.
6. **Sample Diluent:** 1 bottle containing 100 ml of phosphate buffer (10 mM) for sample dilution; pH  $7.2 \pm 0.2$ ; colored yellow; ready to use; white cap;  $\leq 0.0015\%$  (v/v) CMIT/ MIT (3:1).
7. **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB),  $< 0.1\%$ ; ready to use; yellow cap.
8. **Wash Buffer** (20x conc): 1 bottle containing 50 ml of a 20-fold concentrated phosphate buffer (0.2 M), pH  $7.2 \pm 0.2$ , for washing the wells; white cap.
9. **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/L; ready to use; red cap.
10. Cover foil
11. 1 Instruction for use (IFU)

## Materials Required But Not Supplied

1. ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
2. Incubator 37°C
3. Manual or automatic equipment for rinsing Microtiterplates
4. Pipettes to deliver volumes between 10 and 1000  $\mu$ l
5. Vortex tube mixer
6. Distilled water
7. Disposable tubes

## Storage

Store the kit at 2 - 8°C. The opened reagents are stable up to the expiry date stated on the label when stored at 2 - 8°C.

## Specimen Collection And Preparation

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2 - 8°C; otherwise they should be aliquoted and stored deep-frozen (-70...-20°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivation of samples is not recommended.

**Sample Dilution:** Before assaying, all samples should be diluted 1+100 with Sample Dilution. Dispense 10  $\mu$ l sample and 1 ml Sample Dilution into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

## Reagent Preparation

It is very important to bring all reagents and samples to room temperature (20 - 25°C) and mix them before starting the test run!

### Microtiterplate

The break-apart snap-off strips are coated with recombinant Plasmodium antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2 - 8°C.

### Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e.g. 10 ml Washing Buffer + 190 ml distilled water. The diluted buffer is stable for 5 days at room temperature (20 - 25°C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

### TMB Substrate Solution

The reagent is ready to use and has to be stored at 2 - 8°C, away from the light. The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

## Assay Procedure

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µl to 350 µl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout. Select the required number of microtiter strips or wells and insert them into the holder.

- Perform all assay steps in the order given and without any delays.
- A clean, disposable tip should be used for dispensing each standard/control and sample.
- Adjust the incubator to 37 ± 1°C.

### Steps

1. Dispense 100µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1°C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

**Note:** Washing is important! Insufficient washing results in poor precision and false results.

5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.

6. **Adjust tIncubate for 30 min at room temperature (20 - 25°C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20 - 25°C) in the dark.** A blue color occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a color change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

### Measurement

1. Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.
2. If-due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!
3. **Measure the absorbance** of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.
4. Bichromatic measurement using a reference wavelength of 620 nm is recommended.
5. Where applicable calculate the **mean absorbance values** of all duplicates.

### Calculation

#### Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

**Substrate Blank:** Absorbance value **< 0.100**

**Negative Control:** Absorbance value **< 0.200 and < Cut-off**

**Cut-off Control:** Absorbance value **0.150 – 1.300**

**Positive Control:** Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

#### Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations. Example:

Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43, Cut-off = 0.43

#### Results in Units [U]

$(\text{Sample absorbance value} \times 10) / \text{Cut-off} = [\text{Units} = \text{U}]$

Example:  $(1.591 \times 10) / 0.43 = 37 \text{ U}$

#### Interpretation of Results

Cut-off	10 U	-
Positive	> 11 U	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 U	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as <b>negative</b> .
Negative	< 9 U	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

## Performance Characteristics

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 95.88% (95% confidence interval: 91.7% - 98.33%).

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 97.53% (95% confidence interval: 94.97% - 99.0%).

## Precision

Intra assay	n	Mean (E)	CV (%)
#1	24	0.456	10.21
#2	24	1.072	2.74
#3	24	0.931	4.52
Inter assay	n	Mean (U)	CV (%)
#1	12	26.18	5.11
#2	12	22.74	7.20
#3	12	6.17	11.49

## Specificity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal significant evidence of false-positive results due to cross-reactions.

Special attention has to be taken in case of infections with other protozoa like Trypanosoma, Leishmania and Toxoplasma.

## Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.

## Precautions

1. The test procedure, the information, the precautions and warnings in the instructions for use have to be

strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.

2. For research use only.
3. All materials of human or animal origin should be regarded and handled as potentially infectious.
4. All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
5. Do not interchange reagents or Microtiterplates of different production lots.
6. No reagents of other manufacturers should be used along with reagents of this test kit.
7. Do not use reagents after expiry date stated on the label.
8. Use only clean pipette tips, dispensers, and lab ware.
9. Do not interchange screw caps of reagent vials to avoid cross-contamination.
10. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
11. After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
12. To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
13. The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
14. For further internal quality control each laboratory should additionally use known samples.
15. Reagents may contain CMIT/MIT (3:1) or MIT. May cause an allergic skin reaction.
  - Avoid breathing spray.
  - Wear protective gloves/protective clothing.
  - IF ON SKIN: Wash with plenty of soap and water.
  - If skin irritation or rash occurs: Get medical advice/attention.
  - Take off contaminated and Wash it before reuse.
16. Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

## Limitations

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

