Malaria-Ab ELISA

Enzyme Immunoassay for the qualitative detection of antibodies to Plasmodium falciparum, P. vivax, P. ovale and P. malariae in human serum or plasma.

REF RE58601

Σ 96

2-8°C

EU: IVD CE U.S.: For research use only. Not for use in diagnostic procedures.

IBL INTERNATIONAL GMBH
Flughafenstrasse 52a Phone: +49 (0)40-53 28 91-0 IBL@IBL-International.com
D-22335 Hamburg, Germany Fax: +49 (0)40-53 28 91-11 www.IBL-International.com
1 CLINICAL BACKGROUND

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.

This Malaria ELISA kit is designed to detect antibodies occurring in subjects infected with *P. falciparum, P. vivax, P. ovale* and *P. malariae*.

2 INTENDED USE

These kits are intended for use by appropriately trained and qualified personnel for the detection of antibodies to *P. falciparum, P. vivax, P. ovale* and *P. malariae* in human serum and plasma.

3 PRINCIPLE OF THE TEST

The Malaria ELISA 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 and plasma.

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.

4 KIT CONTENTS

<table>
<thead>
<tr>
<th>Plate (96 wells in 12 strips of 8), Polystyrene coated with recombinant antigens</th>
<th>1 (96 tests)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>Human serum</td>
</tr>
<tr>
<td>Negative control</td>
<td>Human serum</td>
</tr>
<tr>
<td>Conjugate</td>
<td>Recombinant antigens conjugated to horseradish peroxidase</td>
</tr>
<tr>
<td>Conjugate dilution buffer</td>
<td>Buffered saline containing surfactant and stabilisers</td>
</tr>
<tr>
<td>Substrate</td>
<td>Urea peroxide and tetramethyl benzidine</td>
</tr>
<tr>
<td>Wash, (20 x concentrated)</td>
<td>Saline containing surfactant</td>
</tr>
<tr>
<td>Stop</td>
<td>0.5M H$_2$SO$_4$</td>
</tr>
</tbody>
</table>

Bag for storing unused wells.

Instruction for use
5 WARNINGS AND PRECAUTIONS

For in - vitro diagnostic use only.
The control materials supplied are derived from human serum. They have been tested at donor level and found negative for Hepatitis B and C, and for HIV 1 and 2. However, they should be treated as if capable of transmitting disease.

Specimens of human serum and plasma should be treated as microbiologically hazardous, and handled in accordance with the applicable regulations.

Do not use the kit after its expiry date.

Do not combine or interchange reagents from kits with different lot numbers.

6 STORAGE

Store at 2 – 8 °C when not in use. Store bottles upright.

Do not freeze.

Do not expose substrate to direct sunlight.

Diluted conjugate is stable for 4 weeks at 4 °C

Diluted wash buffer is stable for 4 weeks at 4 °C

Unused coated strips are stable for 4 weeks at 4 °C if stored in the re-sealable bag provided.

7 EQUIPMENT REQUIRED

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 450 nm and (optionally) at a wavelength between 620 and 690 nm.

37 °C incubator

The 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.

8 SPECIMENS

Serum or plasma (collected into EDTA, sodium citrate, or heparin) specimens should be free of blood cells and of obvious microbial contamination.

They may be stored at 2-8 °C for up to 7 days before testing. Specimens needing longer storage should be frozen at –20 °C or lower.

Frozen specimens should be thawed and well mixed before testing.

9 ASSAY PROTOCOL (MANUAL)

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

Dilute Wash Buffer 1 in 20 with distilled or deionised water 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

Automatic Reading:

Addition of samples is verified by reading at 450nm. A well with sample added will have an A450 reading of between 0.050 and 1.000.
**Addition of conjugate** is verified by reading at 450 nm. A well with sample added will have an $A_{450}$ reading of >0.2.

**Addition of substrate** is verified by reading at 550 nm. A well with sample added will have an $A_{550}$ reading of >0.080.

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

### 9.1 Procedure

1. **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 37 °C for 30 minutes.

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.

3. **Conjugate Incubation**
   - Dilute conjugate $1 + 10$ in Conjugate Buffer. (50 µL + 500 µL per 10 wells)
   - Add **50 µL diluted conjugate** to each well. Incubate (covered) at 37 °C for 30 minutes.

4. **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.

5. **Substrate Incubation**
   - Add **50 µL substrate/chromogen mixture** to each well. Incubate at room temperature for 30 minutes.
   - As the substrate is photosensitive, it is recommended that the plate be protected from light during this incubation.

6. **Stop Colour Development**
   - Add **50 µL stop** to each well. (Blue colour changes to yellow).

7. **Read Results**
   - Read at 450 nm ($A_{450}$). Use of a reference filter at 620 – 690 nm will eliminate effects of scratches, bubbles, etc.

### 10 CUT-OFF VALUE

Calculated as the mean of the negative control values plus 0.100

\[
\text{Cut-Off Value} = \frac{\text{Negative Control 1} + \text{NC2} + \text{NC3}}{3} + 0.100
\]

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

Cut-Off Value = 0.030 + 0.100 = 0.130

### 11 ASSAY VALIDATION

$A_{450}$ 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.

$A_{450}$ of each **Positive Control** should be greater than or equal to 1.000.

### 12 INTERPRETATION

Samples with an $A_{450}$ value less than the Cut-off value are considered **negative** by Malaria-Ab ELISA.

Samples just below the Cut-off (C.O. -10 % $A_{450}$) 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.
13 PERFORMANCE CHARACTERISTICS

13.1 Specificity
External data from 13,608 donor samples deemed at risk to malaria infection gave 96.1 % specificity.
(95 % confidence limits 95.8 % – 96.4 %)

13.2 Sensitivity
External data for 76 acute *P. falciparum* cases showed 92.5 %
(95 % confidence limits 83.6 % - 97.1 %)

External data for 258 IFAT ≥ 80 for *P. falciparum* showed 94.2 %
(95 % confidence limits 90.6 % - 96.7 %)

Internal data for *P. vivax* showed 100 %
(95 % confidence limits 59 % - 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.

13.3 Precision

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>No. of replicates</th>
<th>Mean $A_{450} - A_{620}$</th>
<th>Intra-assay CV (%)</th>
<th>Inter-assay CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>2.402</td>
<td>2.28</td>
<td>3.78</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>1.316</td>
<td>3.83</td>
<td>5.17</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>0.672</td>
<td>3.83</td>
<td>5.52</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>0.353</td>
<td>4.06</td>
<td>6.15</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>0.195</td>
<td>3.19</td>
<td>6.16</td>
</tr>
<tr>
<td>6 (Negative)</td>
<td>16</td>
<td>0.046</td>
<td>6.95</td>
<td>6.84</td>
</tr>
</tbody>
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14 BIBLIOGRAPHY
### Symbols / Symbole / Symbôles / Símbolos / Σύμβολα

<table>
<thead>
<tr>
<th>REF</th>
<th>Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT</td>
<td>Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:</td>
</tr>
<tr>
<td>Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizizzare entro: / Χρησιμοποιείται από:</td>
<td></td>
</tr>
<tr>
<td>CONC</td>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα</td>
</tr>
<tr>
<td>LYO</td>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Λυοφιλιασμένο</td>
</tr>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.</td>
</tr>
<tr>
<td>Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluación. / Kit de avaliação. / Kit di evaluazione. / Кит Аξιολόγηση.</td>
<td></td>
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<tr>
<td>Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell’uso. / Διαβάστε τις οδηγίες πριν την χρήση.</td>
<td></td>
</tr>
<tr>
<td>Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l’abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.</td>
<td></td>
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<tr>
<td>Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:</td>
<td></td>
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<tr>
<td>Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:</td>
<td></td>
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<tr>
<td>Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!</td>
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Symbols of the kit components see MATERIALS SUPPLIED.  
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.  
Voir MATERIEL FOURNI pour les symboles des composants du kit.  
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.  
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.  
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.  
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

### IBL AFFILIATES WORLDWIDE

| IBL International GmbH  
Flughafenstr. 52A, 22335 Hamburg, Germany | Tel.: + 49 (0) 40 532891 -0  Fax: -11 |
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>E-MAIL: <a href="mailto:IBL@IBL-International.com">IBL@IBL-International.com</a></td>
<td></td>
</tr>
<tr>
<td>WEB: <a href="http://www.IBL-International.com">http://www.IBL-International.com</a></td>
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| IBL International Corp.  
194 Wildcat Road, Toronto, Ontario M3J 2N5, Canada | Tel.: +1 (416) 645 -1703  Fax: -1704 |
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**LIABILITY:** Complaints will be accepted in each mode –written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.