**MALARIA TOTAL ANTIBODY EIA**

**CAT. NO.**
60127 Malaria EIA 96 tests
60129 Malaria EIA 480 tests

**KITS FOR THE QUALITATIVE AND SEMI-QUANTITATIVE DETECTION OF ANTIBODIES TO P. FALCIPARUM, P. VIVAX, P. OVALE AND P. MALARIAE IN HUMAN SERUM OR PLASMA BY ENZYME-LINKED IMMUNOASSAY**

**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 two 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 South East Asia and South America.

People infected with *Plasmodium* spp. form antibodies in response. The Lab21 Malaria EIA kit is designed to detect antibodies occurring in subjects infected with *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*.

**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.

**PRINCIPLE OF THE TEST**
Malaria EIA 96 and 480 test 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 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 that 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.

**WARNING 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.

**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.

**EQUIPMENT REQUIRED**
Properly calibrated and maintained pipetting devices capable of delivering volumes of 50µl (specimens and reagents) and approximately 300µl (wash fluids)

Plate or strip reader to read at 450nm and (optionally) at a wavelength between 620 and 690nm

37°C incubator

Lab21 Malaria EIA 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

**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.

**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.
Automatic Reading:

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

**Addition of conjugate:** is verified by reading at 450nm. A well with conjugate added must have an A_{450} reading of >0.2.

**Addition of substrate:** is verified by reading at 550nm. A well with substrate added must 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.

**PROCEDURE**

1. Add 50µl of the undiluted sample (or control – see “Assay Controls” above) to a coated well.
2. Mix on a plate shaker for 30 seconds.
3. Incubate (covered) at 37°C for 30 minutes.
4. Wash 5x with working strength wash buffer. A short soak time of about 30 seconds is recommended between each wash cycle. Tap out excess liquid.
5. Dilute conjugate 1 + 10 in Conjugate Buffer (50µl + 500µl per 10 wells)
6. Add 50µl diluted conjugate to each well.
7. Incubate (covered) at 37°C for 30 minutes.
8. Wash 5x with working strength wash buffer. A short soak time of about 30 seconds is recommended between each wash cycle. Tap out excess liquid.
9. Add 50µl substrate/chromogen mixture to each well.
10. 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.
11. Add 50µl stop to each well (blue colour changes to yellow).
12. Read results at 450nm (A_{450}). Use of a reference filter at 620-690nm will eliminate effects of scratches, bubbles, etc.

**CUT-OFF VALUE**

Calculated as the mean of the negative control values plus 0.100 i.e. Negative Control 1 + NC2 + NC3 + 0.100

Example: 0.030 + 0.025 + 0.035 = 0.030

\[ \therefore \text{Cut-Off Value} = 0.030 + 0.100 = 0.130 \]

**ASSAY VALIDATION**

A_{450} of each Negative Control should be lower than 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

**INTERPRETATION**

Samples with an A_{450} value less than the cut-off value are considered negative by Lab21 Malaria EIA.

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.

**PERFORMANCE CHARACTERISTICS**

**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%).

**Sensitivity**

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

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

Internal data for *P.vivax* showed 100% sensitivity (95% confidence limits 59-100%). Only small numbers of samples from *P.valeae* and *P.malariae* infections have been studied. Sensitivity for these was 80% and 67% respectively. Numbers were too small to allow meaningful statistical analysis.

**BIBLIOGRAPHY**


**KEY TO SYMBOLS**

- IVD In Vitro Diagnostic Medical Device
- Manufactured by
- Temperature limitation
- Use by
- Batch code
- Consult instructions for use
- Coated microtitre plate
- Concentrated wash buffer
- Negative control
- Positive control
- Conjugate
- Conjugate buffer
- Substrate
- Stop solution