DRG® Malaria ELISA (EIA-4263)

Revised 12 Sept. 2011 rm (Vers. 4.1) **RUO in the USA**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 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.

2 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.

3 KIT CONTENTS

R1	**Plate** (96 wells in 12 strips of 8),	Polystyrene coated with recombinant antigens		1 (96 tests)
R2	**Positive control**	Human serum	Red	1.5 mL
R3	**Negative control**	Human serum	Yellow	2.0 mL
R4	**Conjugate**	Recombinant antigens conjugated to horseradish peroxidase	Purple	0.8 mL
R5	**Conjugate dilution buffer**	Buffered saline containing surfactant and stabilisers	Green	8 mL
R6	**Substrate**	Urea peroxide and tetramethyl benzidine	Pink	7 mL
R7	**Wash,** (20 x concentrated)	Saline containing surfactant	Colourless	125 mL
R8	**Stop**	0.5M H_2SO_4	Colourless	7 mL
	Bag for storing unused wells.			
	Directions for use			

4 WARNINGS AND PRECAUTIONS

For research 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.

5 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 4weeks at 4 °C if stored in the re-sealable bag provided.

6 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.

7 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.

8 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 A_{450} reading of between 0.050 and 1.000.

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

Addition of substrate is verified by reading at 550nm. 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

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.

9 BIBLIOGRAPHY

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