# QUANTIMAL™ CELISA

**Ultra-sensitive *Pf*HRP2 Antigen Malaria**

## INTENDED USE AND PRINCIPLE OF THE TEST

The QUANTIMAL™ CELISA Ultra-sensitive *Pf*HRP2 Malaria is a quantitative ELISA based on the original Malaria Ag CELISA assay. Using the same anti-*P.falciparum* (*P.f*) monoclonal antibodies as the Malaria Ag CELISA. This assay was developed to provide improved sensitivity for quantifying HRP-2 to the picogram-level with a lower detection limit at approximately 0.01 ng/mL. Samples of whole blood, culture supernatant, serum or plasma samples and other preparations containing HRP2 can be used. This test can also be used as a confirmatory test for *falciparum* malaria in situations where the traditional diagnosis is unclear, for screening blood transfusion products or to confirm cases of travel-related infection. It is **not** intended to replace the conventional blood film diagnosis.

The sandwich ELISA principle is employed using plastic microwells pre-coated with anti-*P.f* monoclonal capture antibody and a secondary detector anti-*P.f* monoclonal antibody conjugated to horseradish peroxidase enzyme. A sample is added to the microwell and incubated to allow the capture antibody to bind to any HRP2 protein present in the sample. The addition of a conjugated detector IgG antibody forms a binding complex in which a blue colour develops upon the addition of a substrate enzyme. The reaction is stopped by adding a stopping solution that turns the blue complex yellow. The colour intensity is proportional to the amount of HRP2 detected in a sample.

## CONTENTS OF THE KIT

The QUANTIMAL CELISA Ultra-sensitive *Pf*HRP2 is available in 2 different formats:

| | | KM8 | KM8BP |
|---|---|---|---|
| QUMW | CELISA Plate – 1x96 wells - (single use strips) | 2 plates | 10 plates |
| CONTROL + | Standard (freeze-dried) | 1 x 0.5mL | 2 x 0.5 mL |
| CONTROL - | Negative Control | 1 x 5.0mL | 1 x 5.0 mL |
| QUPO | Enzyme Conjugate (200x) | 1 x 0.12mL | 1 x 0.7mL |
| QUCD | Conjugate Diluent | 1 x 24mL | 1 x 120mL |
| QUPT | PBS/Tween (20x) | 1 x 125mL | 2 x 250mL |
| QUSC | Substrate Chromogen (TMB) (20x) | 1 x 1.2mL | 1 x 7mL |
| QUSB | Substrate Buffer | 1 x 24mL | 1 x 125mL |
| QUSS | Stopping Solution | 1 x 12mL | 2 x 30mL |

Store all components at 2-8°C. Important instructions when using the Standard must be noted (see instructions below). Expiry dates are clearly marked on each kit component and on the box. Expiry dates do not change once opened.

## MATERIALS REQUIRED BUT NOT PROVIDED

Malaria positive blood samples, distilled water, micropipettes and tips, clean glassware or plastic containers for solutions, humid chamber, ELISA washer, spectrophotometer to read absorbances at a single wavelength of 450nm, or at dual wavelengths of 450nm and 620nm.

## PRECAUTIONS

For in vitro diagnostic use only. Reagents should not be used after the expiry date shown on the label. If protective packaging is damaged, contact your local distributor and ask for a replacement. Do not mix reagents from different kits. Thimerosal preservative added to some components is a poison. Exercise caution when handling these components. The stopping solution is corrosive. Avoid contact with skin, eyes and mucous membranes. Dispense all reagents with care to avoid cross-contamination of wells. Avoid exposure of the substrate to light. Treat all clinical and control material as though potentially infectious and dispose of in accordance with local operating regulations. For further information, please refer to the Safety Data Sheet.

## INSTRUCTIONS FOR USE

### Preparation of Wash Buffer

If crystals are present, warm the concentrate to dissolve. For each microplate, add 50mL PBS-Tween concentrate QUPT to 950mL of distilled water. Label the bottle WASH BUFFER. Prepare fresh or store pre-prepared buffer in a clean container at 2-8ºC for up to one week if not used immediately.

### Preparation of Samples

Collect patient blood by standard venepuncture procedure using an anticoagulant. Lyse blood using one freeze-thaw cycle to lyse cells and release any HRP-2 antigen present. Use the lysed blood as the test specimen (SAMPLE). High parasitaemia samples detected more than OD 3.0 may be re-tested after diluting to 1:10 or a series of two-fold dilution using RPMI as the diluent. Serum or plasma may be used as an alternative to whole blood although the concentration of HRP-2 is significantly lower compared to whole blood. Blood should be stored at 2-8ºC if analysis is delayed.

### Standard and Negative Controls

The Standard CONTROL + is used to validate the kit performance against the specifications and also for constructing a standard curve for quantification. Keep freeze-dried Standard stored at 2-8ºC along with the kit components. Once ready to use, reconstitute the freeze-dried Standard with distilled water (0.5mL in each vial), leave for 5 minutes and thoroughly mix for a minimum of 1 minute until completely dissolved. Once reconstituted, Aliquot reconstituted Standard in smaller volumes for storage at -20ºC to avoid freeze-thaw cycles. The Negative Control is RPMI. RPMI can be used to dilute the Standard before testing.

**IMPORTANT:** The Standard has been optimised with the ELISA to give an ELISA value (OD) equivalent to the amount of HRP-2, modifications to the assay procedure may change the expected OD values for the Standard at each dilution. The Standard is important in constructing a standard curve to specification and must be handled carefully to ensure Standard integrity is not affected. Refer to page 2 for further information on the quantification method.

### Assay Procedure

1. Bring all reagents to room temperature (18-25 °C) before use.
2. Prepare WASH BUFFER (see Preparation of Wash Buffer)
3. Remove the required number of QUMW strips. Reseal the foil bag containing unused microwell strips and silica gel sachets immediately.
4. To construct a standard curve, prepare a two-fold serial dilution of the reconstituted Standard directly into the microwells. Prepare a 1:10 working stock by diluting 20µL of reconstituted Standard in 180µL of Negative Control CONTROL - in a microtube, mix thoroughly.
5. Pre-load 100uL of Negative Control CONTROL - into 10 microwells, and start the serial dilution by adding 100uL of the 1:10 Working Stock onto the first well, mix gently by pipetting up and down 5 times (5ng/mL). Change pipette tip and continue two-fold dilution onto the next microwell by taking 100µL from the first microwell into the second well etc. up to a maximum of 10 wells.
6. Add 100uL of Negative Control CONTROL - and/or normal whole blood, and test samples into individual microwells. Cover and incubate for one (1) hour at 37ºC in a humid chamber.
7. In the last 10 minutes of the incubation period, prepare the working strength CONJUGATE. The conjugate is 200x concentrate, therefore for each 8-well strip, add 5µL of conjugate concentrate QUPO to 995µL of conjugate diluent QUCD and mix thoroughly. Allow 1mL for each 8-well strip.
8. Wash the wells 4 times, preferably using an automatic plate/strip washer or manual method as follows:
   - *Empty contents from the wells. Refill with the WASH BUFFER.*
   - *Repeat this process a further four (4) times. After the fourth wash, bang inverted wells dry on absorbent tissue.*
   - *NB: take care when flicking out plates, hold side of frame firmly to hold strips in place.*
9. Add 100µL of CONJUGATE to each well. Incubate for 30 minutes at 37ºC in a humid chamber.
10. In the last 10 minutes of the incubation period, prepare the working strength SUBSTRATE. The substrate is 20x concentrate, therefore add 50µL of substrate chromogen QUSC to 950µL of substrate buffer QUSB and mix thoroughly (allow 1mL for each 8-well strip). The stability of the solution is 30 minutes.
11. Repeat washing as in step 8.

12. Add 100µL of fresh SUBSTRATE and incubate in the dark (covered) at room temperature for 15 minutes.
13. Add 50µL of Stopping Solution QUSS. Tap the plate to mix.
14. Read the results in a spectrophotometer at 450nm/620nm wavelength, blanking the machine on air. If dual-wavelength is not available, read at 450nm.

## READING AND INTERPRETATION OF RESULTS AND DIAGNOSIS

***Visually***

Observe the colour intensity of the control and specimen wells 15 minutes after the addition of substrate TMB. The positive results should be blue which turns yellow after the addition of stopping solution. For quantification assays, a spectrophotometer is required for accurate results.

***Photometrically***

Read the microwell plate at 450nm / 620nm (or 450nm if dual-wavelength is not available) in a compatible ELISA plate reader, blanked against air.

For the test results to be accepted the Negative Control must read as follows:

*Table 1: Specifications*

| | **O.D Value (450nm, 450/620nm)** |
|---|---|
| Standard | See Table 2 |
| Negative Control | < 0.1 |
| Cut-Off | = Negative Control OD + 0.1 |

Negative blood samples should give optical density readings below 0.1 OD units. However, to allow for inter-laboratory variation we strongly recommend that each laboratory run a number of known negative blood samples to allow standardisation of the CELISA negative cut-off.

All specimens with an absorbance value above the cut-off level should be considered positive for *P. falciparum* antigen. A positive result indicates the presence of *P. falciparum* antigen. This is suggestive of a current or very recent infection. The intensity of colour is proportional to the level of detected HRP-2. *P. vivax*, *P. ovale* and *P. malariae* infections are not detected. Please note that the test may remain positive for several days after parasites are no longer detectable in blood films.

***Quantification***

It is recommended that the Standard storage and handling be observed due to the sensitive HRP-2. Refer to Standard and Negative Controls section above. The expected Standard Curve is shown below, starting at 10ng/mL. The upper limit (saturation point) of the standard curve is approximately 10ng/mL – 2.5ng/mL with a lower limit of detection at 0.010 ng/mL. For further information, please contact your distributor or contact Cellabs directly at enquiries@cellabs.com.au.

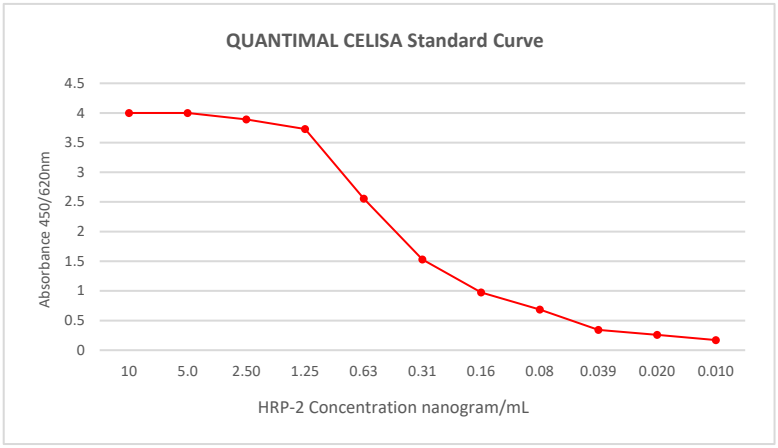


*Table 2: Expected Standard curve OD values*

| HRP2 (ng/mL) | OD |
|---|---|
| 10 | 4.000 (Saturation point) |
| 5 | 4.000 (Saturation point) |
| 2.5 | 3.89 |
| 1.25 | 3.728 |
| 0.63 | 2.554 |
| 0.31 | 1.531 |
| 0.16 | 0.973 |
| 0.08 | 0.686 |
| 0.039 | 0.342 |
| 0.020 | 0.259 |
| 0.010 | 0.171 |

## WASTE DISPOSAL

Dispose of any unused components as biohazardous waste. For more information, please refer to the SDS.

## SENSITIVITY, SPECIFICITY, & OTHER DATA ON THE Quantimal Ultra-sensitive *Pf*HRP-2 CELISA

Refer to summary table at end of insert. All data can be obtained in the product information sheet. Please ask your local distributor or contact Cellabs.

## INDEMNITY NOTICE

Modifications or changes made in the recommended procedure may affect the stated or implied claims. A positive or negative result does not preclude the presence of other underlying causative agents. Cellabs and its agents and distributors shall not be liable for damages under these circumstances.

## FIGURE 1 QUANTIMAL CELISA DIAGRAM FOR USE

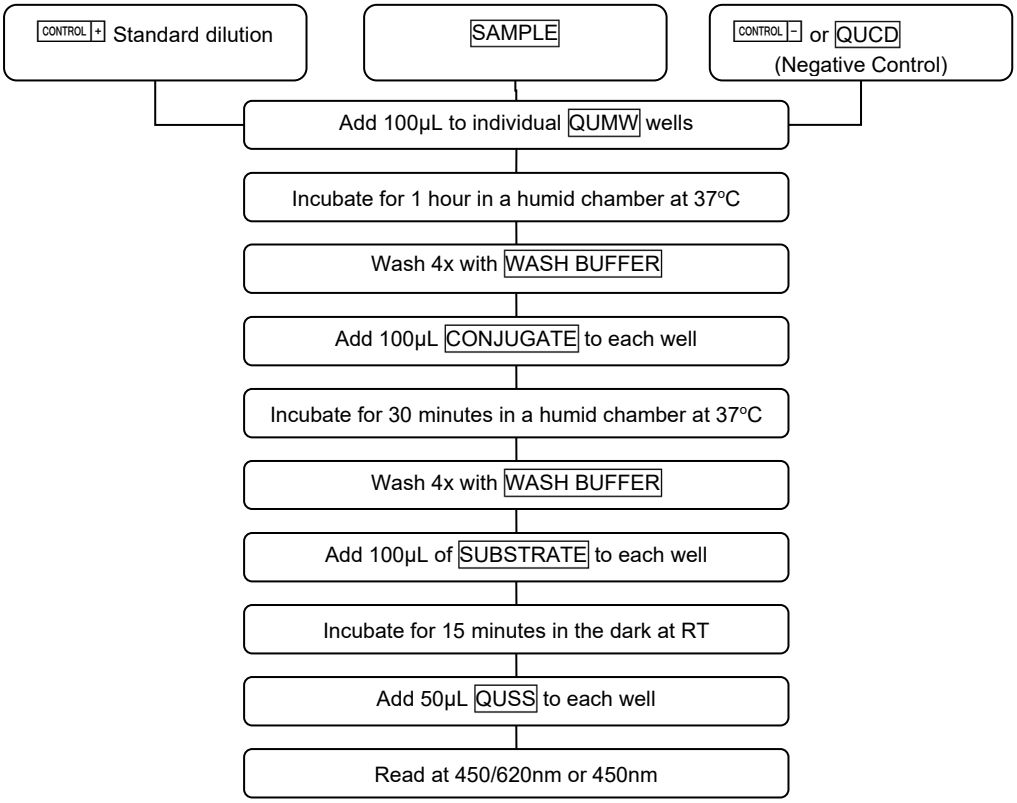


**TABLE 1: SENSITIVITY, SPECIFICITY, & OTHER DATA ON THE QUANTIMAL CELISA**

| **Trial**<br>Essai<br>Versuch<br>Prova<br>Prueba<br>Teste | **Sensitivity**<br>Sensibilité<br>Sensitivität<br>Sensibilita'<br>Sensibilidad<br>Sensibilidade | **Specificity**<br>Spécificité<br>Spezifität<br>Specificita'<br>Especificidad<br>Especificidade | **Repeatability**<br>Répétabilité<br>Wiederholpräzision<br>Ripetibilita<br>Repetibilidad<br>Repetição | **Reproducibility**<br>Reproductibilité<br>Reproduzierbarkeit<br>Riproducibilita<br>Reproducibilidad<br>Reprodutibilidade |
|---|---|---|---|---|
| **A** | 98.1% | 96.2% | - | - |
| **B** | 98% | 96% | - | - |
| **C** | - | - | Positive CV = 5.65% | Positive CV = 9.72% |

## EXPLANATION OF SYMBOLS

Consult Instructions for Use

IVD *In Vitro* Diagnostic Medical Device

2°C 8°C Temperature Limitation

LOT Batch

CONTROL + Control Positive

CONTROL - Control Negative

Use By/Expiration Date

Do Not Re-use

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