English
Product Code: KM8/KM8BP

QUANTIMAL™ CELISA

Ultra-sensitive *Pf*HRP2 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 quantification of HRP-2 to the picogram-level with a lower limit of detection at approximately 0.02 ng/mL. Samples of whole blood, culture supernatant, serum or plasma samples and other preparations containing HRP-2 can be used. This test can also be used as a confirmatory test for *falciparum* malaria in situations where 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 microwells pre-coated with anti-*P.f* monoclonal capture antibody and a secondary detector anti-*P.f* monoclonal antibody conjugated to horseradish peroxidase enzyme. HRP-2 present in a sample binds to the antibody coated on the microwells during an incubation step, a washing step removes the unbound components. The conjugate is added in a second incubation step to allow antibody-HRP-2 to bind, unbound components are removed in a washing step. The antibody-HRP-2 complex is developed for visualisation by the addition of a substrate that turns the wells to a colour blue. The reaction is stopped by adding a stopping solution that turns the blue complex yellow. The colour intensity is proportional to the amount of HRP-2 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 +	Positive Control	1 x 0.5mL	1 x 1.0 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. 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 Material 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. Store at 2-8°C.

Preparation of Samples

Collect patient blood by standard venepuncture procedure using an anticoagulant. Lyse blood using one freeze-thaw cycle, use the lysed blood as the test specimen (SAMPLE). Samples with high parasitaemia that is detected over the limit of detection (> OD 3.0) may be re-tested by titration 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 below -10°C if analysis is delayed.

Positive and Negative Controls

The standardised positive control is used to validate the kit performance against the specifications and to construct a standard curve for quantification. The positive control is not stable at 2-8ºC for more than 20 days after receipt. It is important to store the positive control frozen at -20ºC for short term storage (from date of arrival up to 3 months) or at -80ºC for long term storage (up to 12 months). Aliquoting in smaller volumes before storage is recommended to avoid freeze-thaw cycles. The Negative Control is RPMI. RPMI can be used to dilute the Positive Control before testing.

IMPORTANT: The positive control has been standardised 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 positive control standard curve.

Assay Procedure

1. Bring all reagents to room temperature (18-25 ºC) before use.
2. Prepare WASH BUFFER (see Preparation of Wash Buffer)
3. Remove required number of QUMW strips. Reseal the foil bag containing unused microwell strips and silica gel sachets immediately.
4. Pipette 100µL of each serial dilution of positive control (for constructing the standard curve), test samples and negative controls (RPMI) and/or normal whole blood, into individual microwells. Cover and incubate for one (1) hour at 37ºC in a humid chamber.
5. 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.
6. 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.*
7. Add 100µL of CONJUGATE to each well. Incubate for 30 minutes at 37ºC in a humid chamber.
8. 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.
9. Repeat washing as in step 6.
10. Add 100µL of fresh SUBSTRATE and incubate in the dark (covered) at room temperature for 15 minutes.
11. Add 50µL of Stopping Solution QUSS. Tap the plate to mix.
12. Read the results in a spectrophotometer at 450nm/620nm, 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:

	O.D Value (450nm, 450/620nm)
Positive Control	**>2.0**
Negative Control	< 0.1
Cut-Off level	= Negative Control OD + 0.1

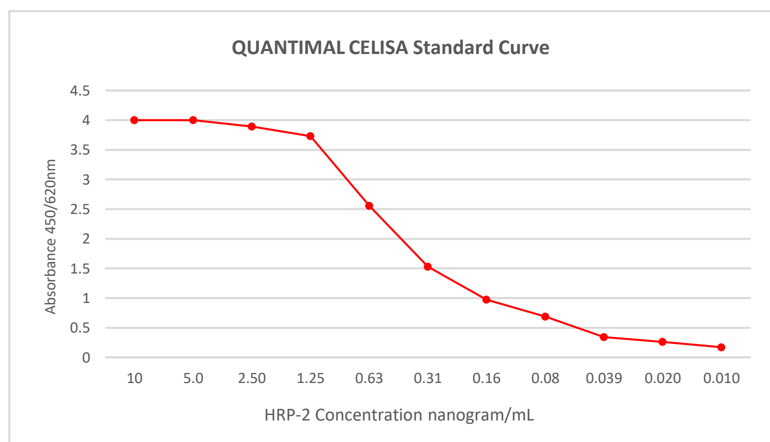
Negative blood samples should give optical density readings below 0.1 OD units. However, to allow for inter-laboratory variation we strongly recommend that each laboratory run a number of known negative blood samples to allow standardisation of the CELISA positive / negative cut-off level.

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 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 positive control storage and handling is observed as HRP-2 is not stable in RPMI at 2-8ºC for more than 20 days. It is also critical to follow the assay procedure as the construction of standard curve is based on these parameters. Any deviation from the assay procedure may not give the correct OD values expected in the standard curve below.

The kit is supplied with extra strips for constructing a standard curve. The positive control is a culture supernatant containing standardised *Pf*HRP2 at a concentration of 100 nanogram/millilitre (ng/mL). To construct the standard curve for quantification, dilute the positive control 1 in 10 in RPMI (100 µL of positive control in 900µL of RPMI) to give a 10 ng/mL concentration. A serial dilution starting at 10 ng/mL (shown as a saturation point in the curve below) is recommended to capture the upper limit and the lower limit of detection. For further information, please contact your distributor or contact Cellabs directly at enquiries@cellabs.com.au.



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 MSDS.

SENSITIVITY, SPECIFICITY, & OTHER DATA ON THE Quantimal Ultra-sensitive *Pf*HRP2 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.

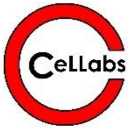


FIGURE 1 QUANTIMAL CELISA DIAGRAM FOR USE

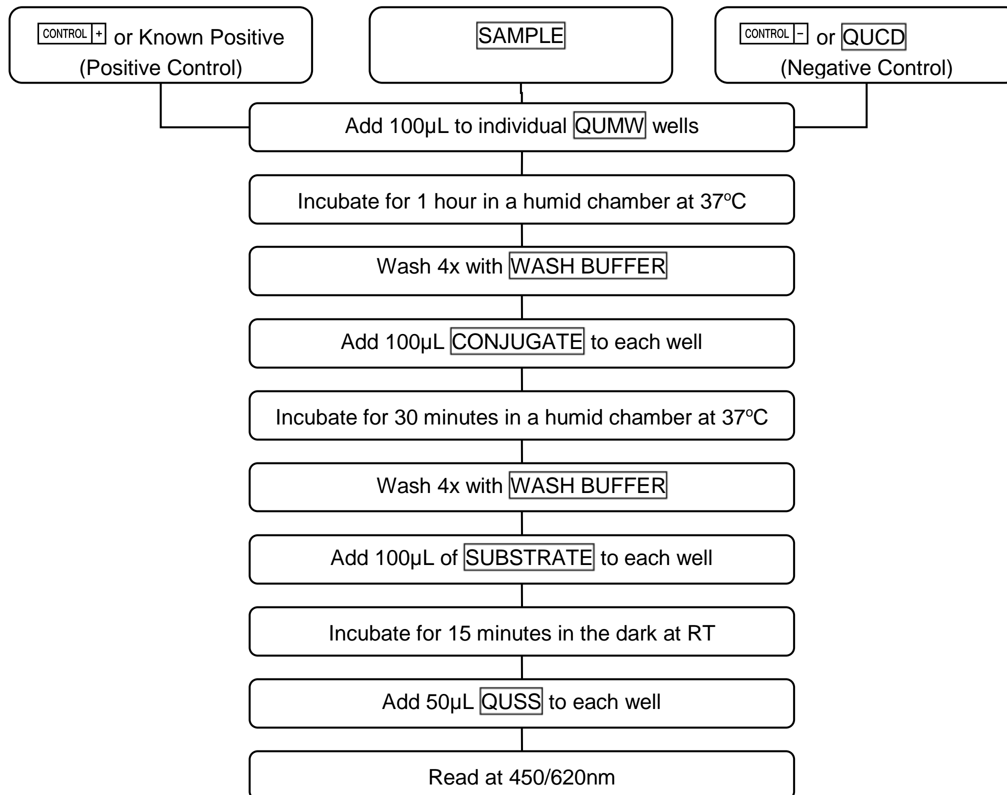


TABLE 1: SENSITIVITY, SPECIFICITY, & OTHER DATA ON THE QUANTIMAL CELISA

Trial Essai Versuch Prova Prueba Teste	**Sensitivity** Sensibilité Sensitivität Sensibilita' Sensibilidad Sensibilidade	**Specificity** Spécificité Spezifität Specificita' Especificidad Especificidade	**Repeatability** Répétabilité Wiederholpräzision Ripetibilita Repetibilidad Repetição	**Reproducibility** Reproductibilité Reproduzierbarkeit Riproducibilita Reproducibilidad 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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