Quantimal™ CELISA
Ultra-sensitive pLDH Malaria

BACKGROUND AND INTENDED USE

The Quantimal™ Ultra-sensitive *p*LDH Malaria CELISA is an enzyme-linked immunosorbent assay (ELISA) for the detection of malarial antigen Plasmodium lactate dehydrogenase (*p*LDH), a glycolytic enzyme present in all five species of human malarial parasites. Since *pLDH* is only secreted by live parasites, levels of this antigen in blood closely reflect the degree of parasitaemia and such, have been found to be a useful indicator of active malaria infection. The Quantimal™ Ultra-sensitive *p*LDH Malaria CELISA is a device for professional use only, it may be used for routine diagnosis, for malaria drug resistance research studies, and for large scale epidemiological screening, monitoring or surveillance.

The sandwich ELISA principle is employed using microwells pre-coated with a monoclonal anti-*p*LDH antibody. A second biotinylated antibody is used as an indicator, followed by a streptavidin conjugate system to form a binding complex if the specific target *p*LDH is present. Washing steps after each incubation period are employed to remove unbound antibody. TMB substrate is added to develop a blue coloured complex in the presence of *p*LDH. The addition of a stopping solution turns the blue wells yellow. The intensity is proportional to the amount of *p*LDH detected in the sample.

CONTENTS OF THE KIT

		KM7	KM7BP
[MPMW]	CELISA plate – 96 ells (single use)	2 plates	10 plates
CONTROL +	Standard (freeze dried)	1 x 0.5 mL	5 x 0.5 mL
CONTROL -	Negative Control	1 x 5.0 mL	1 x 5.0 mL
[MPBA]	Antibody Reagent (200x)	1 x 0.14 mL	1 x 0.7 mL
[MPSA]	SA Conjugate (200x)	1 x 0.14 mL	1 x 0.7 mL
[MPAC]	Antibody and Conjugate Diluent (1x)	1 x 60 mL	1 x 270 mL
[MPPT]	PBS/Tween (20x)	1 x 125 mL	2 x 250 mL
[MPSC]	Substrate Chromogen (TMB) (20x)	1 x 1.2 mL	1 x 7.0 mL
[MPSB]	Substrate Buffer	1 x 24 mL	1 x 125 mL
[MPSS]	Stopping Solution	1 x 12 mL	2 x 30 mL

Store kit at 2-8ºC. Store the positive control frozen using the instructions below. Expiry dates are clearly marked on each kit component and on the box. Expiry dates do not change once opened.

MATERIALS NEEDED BUT NOT PROVIDED

Distilled water, micropipettes and tips, clean glassware or plastic containers for solutions, humid chamber, ELISA washer, ELISA plate reader to read absorbances at a single wavelength of 450nm or at dual wavelength of 450nm and 620nm or automated ELISA systems.

PRECAUTIONS

For in-vitro diagnostic use only. Do not use 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 batch numbers. Thiomerosal preservative added to some components is a poison. Exercise caution when handling these components. The stopping solution is corrosive. Avoid contact with skin, eye and mucous membranes. Ensure all dilution containers are clean before use. 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 (PBS/Tween 20x)

If crystals are present, warm the PBS/Tween 20x bottle to allow crystals to dissolve. For each microplate, add 50mL PBS-Tween 20x [MPPT] to 950mL of distilled water. Label the bottle [WASH BUFFER 1X]. Prepare fresh or store pre-prepared buffer in a clean container at 2-8ºC for up to one week if not used immediately.

Important information on preparation of samples

Red blood cells in whole blood sample must be lysed by a freeze-thaw cycle to release proteins from cells, ensuring the accurate detection of pLDH. After a freeze-thaw cycle, gently mix the sample before testing. Avoid adding blood clots into sample wells. Refer to the **Standard** CONTROL + preparation below. Do not dilute the Negative Control CONTROL - and test samples.

Preparation and collection of blood samples

Collect blood using the standard venipuncture procedure. Use a suitable blood collection tube with anticoagulants such as K2EDTA or K3EDTA. Mix thoroughly. Freeze tubes at -20ºC or -80ºC for a minimum of 30 minutes if testing on the same day or it can be left in the freezer until ready to test. Thaw the blood samples to room temperature and mix well before testing.

Preparation of Standard / Positive Control

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 in smaller volumes for storage at -20ºC to avoid freeze-thaw cycle if not using immediately.

The **Standard** CONTROL + is used as a **Positive Control** to validate the kit performance against the specification or as a **Standard** for constructing a standard curve for quantification. The Standard is provided at 14.0 ng/mL concentration. At this concentration, the OD value in the CELISA system is at a saturated point (OD>3.0). If using the Standard as a Positive Control CONTROL + , dilute 1:1 using the Negative Control CONTROL - or RPMI to achieve a concentration of approximately 7.0 ng/mL (~OD 3.0) before loading onto well. For quantification assays, carefully dilute the Standard in a two-fold serial dilution, starting at neat (14.0 ng/mL), down to 0.11 ng/mL concentration, using one strip (8 wells) or duplicate wells. Use the serial dilution results to construct a standard curve for quantification of pLDH in unknown test samples

ASSAY PROCEDURE

1. Bring all reagents to room temperature (18-25ºC) before use. Note preparation instructions above.
2. Remove required number of plates. Replace unused microwell strips in snap-lock foil bag and seal firmly. Store unused components at 2-8ºC.
3. Bring the pre-prepared wash buffer solution 1x WASH BUFFER working solution to room temperature (refer to Preparation of Wash Buffer (PBS/Tween 20x).
4. Add 100uL of diluted Positive Control or Standards and neat Negative Control CONTROL - in duplicate wells. You may leave a blank well (wash buffer only) to validate non-specific background.
5. Add 100uL of test samples to designated wells.
6. Incubate plate in a humidity chamber for 30 minutes at 37ºC. A humidity chamber can be created in a plastic box with a lid. Place a few layers of laboratory tissue at the bottom of the plastic box and place the plates in the box with the lid on.
7. Ten minutes before the end of the first incubation period, prepare to dilute the 200x Antibody Reagent [MPBA]. For each strip (8 wells), prepare 1mL of 1x working solution by diluting 5uL of Antibody Reagent Concentrate [MPBA] in 995uL of Antibody and Conjugate Diluent [MPAC]. Mix thoroughly.
8. Wash the wells five times (5x) using the 1x WASH BUFFER. Tap the plate on laboratory tissue to remove excess buffer.
9. Add 100uL of working solution 1x Antibody Reagent to each well and incubate in a humidity chamber for 30 minutes at 37ºC.
10. Ten minutes before the end of the second incubation period, prepare to dilute the 200x SA Conjugate [MPSA]. For each strip (8 wells), prepare 1mL of 1x working solution by diluting 5uL of SA Conjugate in 995uL of Antibody and Conjugate Diluent [MPAC].Mix thoroughly.
11. Wash the wells five times (5x) using the 1x WASH BUFFER. Tap the plate on laboratory tissue to remove excess buffer.
12. Add 100uL of working solution 1x SA Conjugate to each well and incubate in a humidity chamber for 30 minutes at 37oC.
13. Wash the wells five times (5x) using the 1x WASH BUFFER. Tap the plate on laboratory tissue to remove excess buffer.
14. Prepare the working solution 1x Substrate. For each strip (8 wells), prepare 1mL of 1x Substrate by diluting 50uL of 20x Substrate Chromogen (TMB) [MPSB] in 950uL of Substrate Buffer [MPSB]. Mix thoroughly.
15. Immediately add 100uL of working solution 1x Substrate into each well and incubate in the dark at room temperature for 10 minutes.
16. After the incubation period, immediately add 50uL of Stopping Solution [MPSS] to each well. Gently tap the sides of the plate to mix.
17. Read Results using a plate reader at single wavelength 450nm or dual wavelength 450nm/620nm.

ASSAY PROCEDURE FOR AUTOMATED SYSTEMS
Each automated ELISA robotic system is different. This ELISA shall be evaluated to determine the correct parameters to use in each ELISA robotic system. Use the following procedure as a guide only.

Preparation for Testing

1. Bring all reagents to room temperature (18-25ºC) before use.
2. Remove required number of plates. Replace unused microwell strips in snap-lock foil bag and seal firmly. Store at 2-8ºC.
3. Prepare the standard to be used as a Positive Control for automated testing. Refer to Preparation of Standard / Positive Control.
4. Bring the pre-prepared wash buffer solution 1x WASH BUFFER working solution to room temperature. Refer to Preparation of Wash Buffer (PBS/Tween 20x).
5. Preparation of Antibody Reagent [MPBA]. For each plate, prepare 12 mL of 1x Antibody Reagent by diluting 60uL in 11.94 Antibody and Conjugate Diluent [MPAC].
6. Preparation of Streptavidin Conjugate [MPSA]. For each plate, prepare 12mL of 1x Streptavidin Conjugate by diluting 60uL in 11.94 Antibody and Conjugate Diluent [MPAC]
7. Ensure lysed whole blood samples, controls and reagent are ready for loading.
8. For automated systems, initiate machine to prepare for the assay. The automated machine shall be configured as below.

Automated System Parameter Procedure

1. Into the designated control wells, add 100uL of positive and negative controls in duplicates. Include duplicate blank wells (no sample).
2. Add 100 µL each of lysed blood sample into the designated test wells.
3. Incubate the plates in a humidity chamber for 30 minutes at 37ºC.
4. Wash the wells five times (5x) using pre-diluted 1x working wash buffer (PBS/T).
5. Add 100 µL of pre-diluted Antibody Reagent into each well and incubate in a humidity chamber for 30 minutes at 37ºC
6. Wash the wells five times (5x) using pre-diluted 1x working wash buffer (PBS/T).
7. Add 100 µL of pre-diluted working strength SA Conjugate to each well and incubate in a humidity chamber for 30 minutes at 37ºC.
8. Wash the wells five times (5x) using pre-diluted 1x working wash buffer (PBS/T).
9. Prepare the 1x working strength SUBSTRATE. Allow 12.0mL for each plate (mix 0.6mL of concentrated SUBSTRATE in 11.4mL SUBSTRATE DILUENT
10. Add 100 µL of pre diluted substrate and incubate in the dark at room temperature for 10 minutes.
11. Add 50 µL of Stopping Solution [MPSS]. Gently shake to mix.
12. Read the results at single wavelength of 450nm or dual wavelength at 450nm/620nm.

READING AND INTERPRETATION OF RESULTS AND DIAGNOSIS

Visually
Observe the colour intensity of the control and specimen wells. The Standards/Positive Controls should be blue before, and yellow after stopping.

Photometrically
Read the microwell plate at single wavelength 450nm or dual wavelength 450/620nm in a compatible ELISA plate reader, blanked against air. For the test results to be accepted, the control must read as follows:

	Manual Operation 450nm or 450/620nm	**Automation 450/620nm**
Negative Control	O.D < 0.2	OD < 0.05
Positive Control (Diluted ½)	O.D > 2.0	OD > 2.0
Blank	O.D < 0.2	OD < 0.05
Cut-off value (COV)	O.D 0.25 (OD 0.3 at 450nm)	OD 0.05*

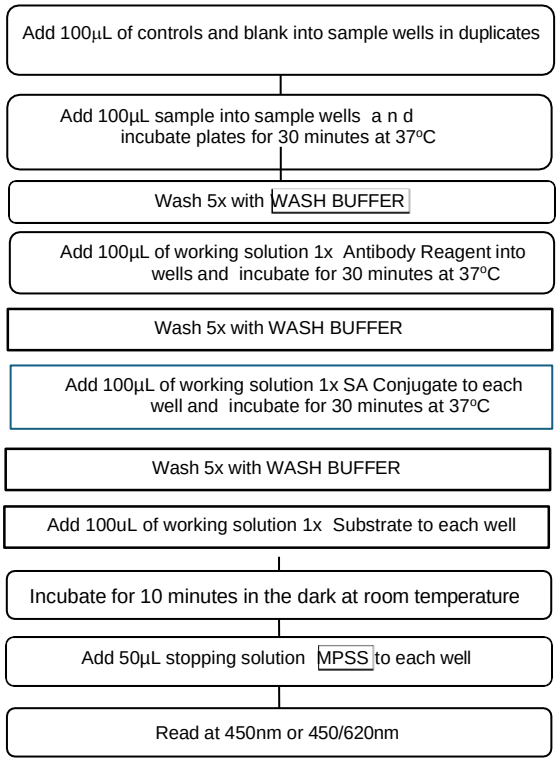
Cut off value (COV) for manual or semi-automatic operation was based on the OD values from whole blood samples from a non-endemic population. A baseline may be determined by testing a minimum of 30 whole blood samples from a non-endemic population. Equivocal results shall be carefully repeated.
**This, COV was determined using the Dynex Agility Automated System. COV for other automated open systems may differ in optical system. It is a requirement to optimise the parameters of automated systems to determine the correct COV for use. Please contact Cellabs technical team to determine the correct COV for your application.*

A positive result indicates the presence of pLDH from *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* or *Plasmodium knowlesi*. The Quantimal™ Ultra-sensitive pLDH Malaria CELISA does not distinguish between different species of *Plasmodium* infections. To detect specific infection caused by *Plasmodium falciparum,* contact Cellabs Pty Ltd for information on the Malaria Antigen (HRP-2) CELISA or Quantimal Ultra-sensitive PfHRP2 CELISA.

WASTE DISPOSAL
Dispose of any unused component as bio-hazardous waste. For more information, please refer to the SDS.

INDEMNITY NOTICE
Modifications or changes made in the recommended procedure may affect the stated or implied claims and performance of the kit. Cellabs and its agents or distributors are not liable for damages under these circumstances.

FIGURE 1: Quantimal Ultra-sensitive pLDH Malaria CELISA Method Summary



EXPLANATION OF SYMBOLS

	Consult Instructions for Use
IVD	*In Vitro* Diagnostic Medical Device
2℃ 8℃	Temperature Limitation
LOT	Batch
CONTROL +	Control Positive
CONTROL −	Control Negative
	Use By/Expiration Date
	Do Not Re-use

Cellabs Pty Ltd
Unit 7, 27 Dale Street
Brookvale, NSW 2100 Australia
Tel: +61 2 9905 0133 Fax: +61 2 9905 6426
Web: http://www.cellabs.com.au
Email: sales@cellabs.com.au

EC REP WMDE B.V
Bergerweg 18
6085 AT Horn
The Netherlands

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