Malaria Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAMAL1	Malaria Elisa	96 Tests

Intended Use:

Malaria Elisa is intended to be used for the qualitative detection of Malaria specific antigen (pLDH) in human whole blood samples. For In Vitro Diagnostic use only.

Summary and Principle:

Four species of Plasmodium parasite are responsible for malaria infection in humans viz. *P. falciparum, P.vivax, P.ovale and P.malariae*. Malaria Elisa detects the presence of malaria genus specific pLDH released by parasite blood cells. Since pLDH is produced by viable parasites, the assay can also be used to monitor success of anti-malarial therapy. Malaria Elisa is especially designed to exclude infected blood from the blood supply to prevent transfusion. Acquired malaria.

A monoclonal antibody pan-specific for all species of pLDH is coated onto the wells of the microtiter strips. Samples are pipetted into the wells for binding to the immobilized antibody. After extensive washing to remove unbound material, pLDH is recognized by the addition of a biotinylated monoclonal antibody also pan-specific for pLDH. After removal of excess biotinylated antibody, streptavidin-peroxidase is added. Following a final washing, peroxidase activity is quantified using the substrate solution based on 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H2O2). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of pLDH in the samples.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Microwells are coated with monoclonal anti-p-LDH antibodyThe microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable for 1 month at 2-8°C.
Positive Control	1x1ml	Goat-anti mouse serum with stabilizer. Produces a positive reaction. Ready to use. Once open stable for 3 months at 2-8°C.
Negative Control	1x1ml	Bovine Serum Albumin with stabilizer. Produces a negative reaction and is used for cut-off calculations. Ready to use. Once open stable for 3 months at 2-8°C.
Enzymatic Conjugate	1x0.5ml	Streptavidin-HRP conjugate (50X). To be diluted 50 times with conjugate diluent. Once open stable for 3 months at 2-8°C.
Conjugate Diluent	1x30ml	Buffered solution containing stabilizing proteins and preservatives. Once open stable for 3 months at 2-8°C.
Antibody Reagent	1x0.5ml	Biotinylated anti-pLDH antibody (50X). To be diluted with conjugate diluent. Once open stable for 3 months at 2-8°C.
Sample Diluent	1x15ml	Buffered solution containing stabilizing proteins and 0.1% sodium azide as preservative. Once open stable for 3 months at 2-8°C.
Substrate	1x12ml	Solution containing Tetramethyl benzidine (TMB) and hydrogen peroxide. Ready to use. Store at 2-8 °C in a closed container protected from direct light. Stable until the expiry date.
Wash Buffer	1x60ml	Buffer containing surfactants (20X). To be diluted 20 times with distilled or deionized water. Store at 2-8 °C. Stable until the expiry date.
Stop Solution	1x15ml	Diluted Sulphuric acid (0.3N). Store at 2-8 °C. Stable until the expiry date.

Working Washing Solution diluted 1:20 has to be kept in room temperature for max. of 1 week.

Plastic Sealable bag, IFU and Plate sealer.

Materials required but not provided:

Manual or automatic pipette, pipette tips, incubator, absorbent sheets, Elisa washer, Elisa reader, troughs or boats, disinfectant, reagent grade water, disposable gloves, timer, biohazard waste container, serological pipettes.

Specimen Collection:

1. No prior preparation of the patient is required
2. Collect blood specimen by venepuncture according to the standard laboratory procedure
3. Specimen should be free of particulate matter and microbial contamination
4. Preferably use fresh whole blood sample. However, specimen can be stored refrigerated at 2-8 °C up to three days. Specimen should be kept frozen at -20°C or lower, if sample storage is required for longer duration
5. Specimens should be brought to room temperature prior to testing
6. Anticoagulants (Heparin, EDTA or Citrate) don't interfere with the test results

Precautions:

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with Sodium hypoclorite.
7. Dispose off all the reagents and material used as if they contain infectious agent.
8. Neutralize acid containing waste before adding hypoclorite.
9. Sample diluent contains Sodium azide; avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build-up in the plumbing.
10. Do not use kit after the expiry date.
11. Do not mix components of one kit with another.
12. Always use new tip for each specimen and reagent.
13. Do not allow liquid from one well to mix with other wells.
14. Do not let the strips dry in between the steps.
15. Mix blood sample before taking a specimen for testing.

Reagent Preparation:

1. Dilute wash buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water).
2. Dilute antibody reagent 50 times (for example add 20µl reagent to 980µl conjugate diluent).
3. Dilute enzyme conjugate 50 times (for example add 20µl concentrated enzyme conjugate to 980µl conjugate diluent).

Procedure:

STEP 1

Preparation: Bring all the reagents and specimen to room temperature before use. Take out required number of strips and immediately close the pouch. Prepare data sheet indicating the location of controls and specimen. Use controls in duplicate.

STEP 2

Addition of control, whole blood and sample diluent: Add 25µl control or whole blood specimen in spate wells, except A1 well. Add 100µl Sample diluent in each wells except A1 well. Gently shake the plate to mix contents.

STEP 3

Incubation: Apply plate sealer and incubate for 30 minutes at 37°C.

STEP 4

Washing: Wash each well by filling approximately 350 µl diluted wash buffer, giving 30 seconds soak time for each wash and aspirating/flicking off six times. Blot dry.

STEP 5

Addition of Antibody reagent: Add 100µl diluted antibody reagent in each wells, except A1 well and incubate at 37°C for 30 minutes.

STEP 6

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with diluted washing buffer of 350ul. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 7

Addition of the Substrate: Add 100ul of Substrate Solution to each well. Mix gently for 5 seconds.

STEP 8

Washing: Wash six times as in step 6. Blot dry.

STEP 9

Addition of Substrate: Add 100µl of substrate in each well, including A1 well and incubate at room temperature (20-28°C) away from light for 30 minutes.

STEP 10

Stop reaction: Stop reaction by adding 100µl of Stop solution in each well, including A1 well. The stop solution should be added in the same sequence as substrate addition.

STEP 11

Measurement: Read the absorbance of each well at 450nm with 600-700 nm as reference with 30 minutes of stopping reaction.

Run criteria:

1. The individual absorbance value of negative controls should be less than 0.1.
2. The individual absorbance value of positive controls should be more than 1.0.
3. If the test end does not meet above criteria, test run is invalid and should be repeated.

Calculation of results:

The absorbance of 'Blank well' should be subtracted from the absorbance of test samples and controls.
The cut-off value (COV) is calculated by adding 0.1 to average absorbance value of negative control.
Example: **COV=Av.NC+0.1**

Absorbance of Negative Control (NC)		Corrected Absorbance (Abs-blank)
Reading – 1	0.03	0.021
Reading – 2	0.027	0.018
Average NC reading		0.019

Absorbance of Positive Control (PC)		Corrected Absorbance (Abs-blank)
Reading – 1	2.823	2.814
Reading – 2	2.807	2.798
Average PC reading		2.806

Well	Absorbance	Corrected Absorbance (Abs-Blank)	Mean	Cutoff	Result
Blank	0.009				
NC	0.03	0.021	0.019	0.019	
NC	0.027	0.018			
PC	2.823	2.814	2.806		
PC	2.807	2.798			
Sample 1	3.487	.478			Reactive
Sample 2	0.654	0.645			Reactive
Sample 3	0.056	0.047			Non-Reactive

Interpretation of Results:

1. Samples with absorbance values less than the cutoff value are considered non-reactive by Prestige Malaria Elisa kit and are considered negative for malaria.
2. Samples with absorbance value equal to or greater than cutoff value are considered reactive by Prestige Malaria Elisa kit. The original sample should be retested in duplicate. Initially reactive sample that do not react in either of duplicate are considered negative for malaria.
3. If a sample is repeatedly reactive the probability of malaria infection are high, especially with patients at high risk or high absorbance values. Such samples should be retested with microscopy of thick smear and thin blood films.
4. In case of samples with high OD, there are possibilities of black precipitate formation after the addition of stop solution. This will not interfere with the interpretation of results.

Performance characteristics:

In an in-house study, a panel of 282 samples whose results were confirmed with microscopy was tested with Malaria Elisa.
The results are shown in the table below:

Samples	Total No. tested	Prestige Malaria Elisa		Sensitivity	Specificity
		Positive	Negative		
P.falciparum	43	43	0	100%	
P.vivax	25	25	0	100%	
P.ovale	2	2	0	100%	
P.malariae	2	2	0	100%	
Malaria Negative	210	0	210		100%

Limitation of the Procedure:

Malaria Elisa Kit alone cannot be used to diagnose malaria infection even if the sample is repeatedly reactive or has high absorbance value. A physician can only establish clinical diagnosis. A negative result does not preclude the possibility of exposure to or infection with malaria.

References:

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REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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