Erba Mannheim

ErbaLisa® PAN (LDH) MALARIA

96 tests **Product Code: 130768**

FOR THE QUALITATIVE DETECTION OF MALARIA SPECIFIC ANTIGEN (PLDH) IN HUMAN WHOLE BLOOD

OVERVIEW

Malaria is one of the major infectious diseases across the world in terms of mortality. It is caused by the parasite Plasmodium, which spreads as a result of mosquito bite and is characterized by fever, chills and anemia. Malaria is caused by four species of Plasmodium- P. falciparum, P. vivax, P. ovale and P. malariae. About 500 million cases of malaria infection are reported globally every year, resulting in about 3 million deaths worldwide, mainly due to P. falciparum. Children below five years and pregnant women are at greater risk of complications and death due to P. falciparum infection which shows large-scale drug resistance

INTENDED USE

ERBA LISA® PAN (LDH) Malaria uses monoclonal antibodies to LDH of all Plasmodium species (pLDH) as coating material. Since LDH is a product of viable parasites, it can be used to monitor antimalarial therapy.

PRINCIPLE

The **ERBA LISA® PAN (LDH) Malaria** test kit is a solid phase immunoassay for the qualitative detection of pLDH from four Plasmodium species in human blood.

Addition of positive control or pLDH containing human blood will form a stable complex with the bound antibody present in the well. After washing, a mixture of monoclonal antibodies against pLDH conjugated with HRPO is added to the wells. Only the bound antigen-antibody complex present in the well will react with the conjugate. A washing step will remove the unbound conjugate molecule. Addition of color reagent will develop blue color only in positive control wells and wells containing pLDH in test specimen. Upon addition of stopping solution, blue color changes to yellow. The intensity of developed yellow color is directly proportional to the presence of bound pLDH in the respective wells.

KIT CONTENTS

Sr. No.	Reagents	Presentation
1	***Coated Microwells*** – Breakaway microwells coated with Anti-PAN LDH antibodies	8 wells x 12 strips
2	***Sample Diluent PL*** – Ready to use, buffer with animal protein and detergent	12.0 mL
3	***PAN LDH Negative Control*** – Ready to use, buffer with animal protein and detergent	1.0 mL
4	***PAN LDH Positive Control*** – Ready to use, procedural positive control buffer containing Goat Anti-mouse serum	1.0 mL
5	***Conjugate PL***– Ready to use, monoclonal anti-pLDH conjugated with horseradish peroxidase	6.0 mL
6	***Washing Solution (20X conc.)*** – Buffer containing surfactant. Dilute 1:20 with distilled water before use	30.0 mL
7	***Color Reagent*** – Ready to use, 3,3',5,5'-Tetra methyl benzidine, Dimethyl sulfoxide, H_2O_2	6.0 mL
8	***Stopping Solution*** – Ready to use, phosphoric acid	12.0 mL
9	***Black Plate Cover*** – To avoid exposure to light during incubation	1
10	***Strip Sealers*** – To cover strips during incubation in order to avoid evaporational loss	2

***Items 1 to 8 should be stored at 2-8°C.**

MATERIALS REQUIRED BUT NOT PROVIDED

- Absorbent paper
- Disposable gloves and protective glasses
- Pipettes capable of delivering 0-20 & 50-200µl volumes
- Disposable tips
- Graduated cylinder
- Waste disposal container
- Timer
- ELISA reader (**Erba Lisa Scan®**)
- Automatic / Semi-automated washing system (**Lisa Wash®**)
- Distilled / deionized water
- Sodium hypochlorite solution for disposal of waste

STORAGE

- The shelf life of the kit is 18 months from the date of manufacturing when stored at 2-8°C.
- **Do not FREEZE the components.**
- Immediately after use, return all reagents to 2-8°C.
- The reconstituted wash buffer is stable for 2 months when stored at 2-8°C.
- The unused microwell strips are suitable for use for 4 weeks after opening the foil pouch when stored at 2-8°C with desiccant in the Ziploc pouch.

PRECAUTIONS

- DO NOT perform the test in case there is a cut or wound in the hands.
- Disposable gloves should be worn throughout the procedure.
- Before use, wait for all reagents to stabilize to room temperature.
- DO NOT use kit components beyond expiration date as indicated on the labels.
- DO NOT mix reagents between different lots as these are optimized for individual batch performance.
- All specimens and controls should be considered potentially infectious and discarded appropriately.
- Use a new tip for each controls and specimen.
- Use dedicated disposable tips to avoid microbial contamination of the reagents.
- Use clean glassware rinsed with distilled water.
- Use only distilled / deionized water to reconstitute the washing solution.
- DO NOT interchange caps of the reagents.
- Run the recommended number of positive and negative controls in each assay to ensure validity of the kit.
- Use whole blood only, collected in anti-coagulant agent.
- DO NOT allow the microplate wells to dry between the end of washing and the addition of the reagent.
- Incubation time should not vary by more than ± 2 minutes.
- After using required number of strips, rest of the strips along with activated silica gel should be kept in sealed condition into ziplock bag.
- DO NOT expose the color reagent to light.
- Level the microwell strips in the holder before reading the results. Wipe the bottom of the wells with a clean tissue to remove any dust or moisture.
- The test is for in-vitro diagnostic use and should be performed and interpreted by a competent or trained person only.
- Wash hands thoroughly with a suitable detergent, after the use of kit.
- In case of an accident, such as contact with eyes or contact of contaminated material with skin wounds or ingestion of contaminated material, etc. consult a physician immediately.
- Spills should be immediately decontaminated with a suitable disinfectant.
- Prior to disposal, all waste material should be collected and soaked in 5% sodium hypochlorite for at least 30 minutes.

SPECIMEN COLLECTION AND STORAGE

ERBA LISA® PAN (LDH) Malaria is recommended to be used only for testing of human whole blood samples. Collect the specimen aseptically. Whole blood should be collected in presence of an anticoagulant like EDTA, Heparin or Citrate. **Mix properly by inverting the tube up & down before use.**

Samples can be stored at 2-8°C for a week or frozen at -20°C until use. Frozen specimen should be completely thawed before use.

RECONSTITUTION OF REAGENTS

Dilute washing solution 1:20 in distilled or deionized water. Homogenize. Washing solution may form crystals under cold storage conditions. If so, use it after thawing at 37°C in a water bath.

ASSAY PROCEDURE

It is recommended that the assay procedure should be strictly adhered to obtain reliable results.

1] 1 Bring all the reagents and test specimens to room temperature and shake well before use.
2] Define the sample / control distribution and identification plan. In each run, assign one well for the Blank (A1), 3 wells for the PAN LDH negative control (B1, C1, D1) and 1 well for the PAN LDH positive control (E1).
3] Break the number of required wells for a run. Wrap the balance unused wells tightly in zip-lock pouch with desiccant and return it to 2-8° C immediately.
4] Add 100 µL of the Sample Diluent PL in well A1 (blank) & Test Sample wells (F1, G1 and so on…) no sample Diluent in control wells
5] Add 100 µL of PAN LDH negative control in wells B1, C1 and D1 & positive control in well E1.
6] Add 10 µL of the first sample in well F1, second sample in well G1 and so on….
7] Mix well, cover the wells with the strip sealers and incubate for 45 minutes at 37°C.
8] Remove the sealer. Discard / aspirate the contents of the well into the waste disposal container. Add a minimum of 350 µL of washing solution to each well. Aspirate again after 30 seconds of soak time. Repeat the washing step 5 times (Invert the plate and tap it on absorbent pad to remove the remaining washing solution).
9] Add 50µl Conjugate PL into all the wells including in well A1 (blank). Mix well, cover the wells with the strip sealers and incubate for 30 minutes at 37°C.
10] Repeat Step 8
11] Add 50µl of the color reagent to all wells including well A1 (blank). Cover the plate with the black cover provided and allow the reaction to develop in the dark for 15 minutes at room temperature (20-30°C).
12] Add 100 µl stopping solution to all wells. Homogenize. After the addition of the stopping solution the blue color of the substrate turns to yellow (for positive samples) or remains colorless (for negative samples).
13] Carefully wipe the plate bottom and read the optical density at 450 nm (using 620/630/650 nm as the reference wavelength). **Deduct Blank absorbance from the control and test wells.**

CALCULATION AND INTERPRETATION OF RESULTS

Blank value : Absorbance value of blank should be less than 0.1
Positive Control : Absorbance value of the positive control should be greater than 0.5
Negative Control : Absorbance values of the individual negative controls should be less than 0.1 after subtracting from the respective OD's, the Blank OD.
Calculate the mean of the measured absorbance values for the PAN LDH Negative Control (Ncx)

Calculation of NCx:

Example :

NC	Absorbance
B1	0.027
C1	0.030
D1	0.034

NCx = (0.027+0.030+0.034) / 3 = 0.030
COV = 0.1 + 0.030 = 0.130

Calculations of the Cut-Off Value (COV)

COV = 0.1 + NCx

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Interpretation of the result:
Non-Reactive: If the absorbance of the test serum is less than the cut-off value, then the sample is considered as non-reactive.
Reactive: If the absorbance of the test serum is equal or greater than the cut-off value, then it is considered as initial reactive. This sample should be retested in duplicates. If the absorbance of duplicate retest results is less than cut-off value, then the specimen is considered as non-reactive. If both the duplicate retest results are found reactive, then the specimen is considered as repeatedly reactive.
Repeatedly Reactive specimens found in **ERBA LISA® PAN (LDH) Malaria** must be further confirmed with other tests like microscopy and by PCR.

PERFORMANCE CHARACTERISTICS

The performance of **ERBA LISA® PAN (LDH) Malaria** has been determined in-house using a panel containing 210 positive samples and 820 negative clinical samples.

The results of the in house study are as follows:

Sample Description	ERBA LISA® PAN (LDH) Malaria	
	Reactive	Non-Reactive
PAN LDH Positive (210)	210	0
PAN LDH Negative (820)	0	820

Sensitivity – 100% **Specificity – 100%**

Note:
1. Using Erba Lisa Kits, in some instances, it is possible to obtain OD values of controls and samples preceded by a negative sign. An OD up to -0.05 is acceptable and can be considered Zero OD for calculation of the cut-off value. This will, in no way, affect the assay performance or the test results.
2. The ELISA readers have a linear measuring range approximately 2.5A. Beyond this range OD values are non-linear. Therefore many instruments programmed to show 'OUT' or 'OUT OF RANGE' indication, if the OD exceeds 2.5 or 3 or more, even after dilution of sample or control. Please note it indicates a valid run provided the negative control value is <0.1 and does not interfere with assay results.
3. If any sample/control gives a negative OD value after deduction of blank, consider the value as 0 (Zero).

LIMITATIONS OF THE TEST

- **ERBA LISA® PAN (LDH) Malaria** is a screening test. All reactive samples should be further confirmed by supplemental assays such as microscopy, PCR etc.
- A non-reactive test does not exclude the possibility of Plasmodium infection.
- The assay is only valid for human whole blood and not for pool of samples or other body fluids or animal blood products.
- In case the kit is not stored properly or the test is not performed as per the recommended instructions, it may lead to erroneous results.

LIMITED EXPRESSED WARRANTY DISCLAIMER

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For IVD use only

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ErbaLisa® PAN (LDH) MALARIA

Add 100ul of Sample Diluent PL in Blank & Sample Wells
(No sample diluent in control wells)

Add 100ul of Controls in respective wells
Add 10ul of Sample in respective wells
(B, 3NC, 1PC, S1, S2, ….)

Mix properly & Incubate at 37°C for 45 min

Wash all the wells 6 times using 350ul washing solution per well

Add 50ul of Conjugate PL to each well

Mix properly & Incubate at 37°c for 30 min

Wash all the wells 6 times using 350ul washing solution per well

Add 50ul of Color Reagent to each well

Mix properly & Incubate at RT for 15min in Dark

Add 100ul of Stop Solution to each well

Read the absorbance using 450nm
(with reference to 620nm)

Trouble Shooting Guide

Query	Possible Cause	Explanation / Solution
Control OD out of validation limit / Invalid results	a) Incorrect incubation timing or temperature b) Incorrect pipetting volume c) Improper mixing of specimens / reagents d) Cross contamination e) Use of reagents from different batches f) Use of expired reagents g) Washing errors	a) Check procedure / Repeat the assay b) Keep a check on pipetting technique and volume aspirated/dispensed c) Repeat the assay with proper mixing d) Ensure use of fresh tips for every dispensing carried out e) Not recommended as reagents are standardized for the individual batch released f) Use reagents within its shelf life mentioned on the vials. g)Strictly adhere to the wash procedure detailed in the package insert; check the wash buffer container, quality and pH of the water used for dilution
High absorbance value of negative control (color development in negative control wells)	a) Contamination of reagents / control b) Cross contamination c) Insufficient washing-filling volume insufficient / insufficient wash cycles d) Drying of wells during wash cycles.	a) Do not interchange caps of controls / reagents. Repeat the assay b) Avoid splashing between wells during pipetting and washing c) Strictly adhere to wash procedure detailed in the package insert. d) The wells should not be dried intermittent to the wash cycles. After washing blot the micro plate/wells on tissue paper & tap properly.
Low absorbance value of positive control (no color or faint color at the end of the assay)	a) One of the reagents missed out or added in wrong sequence b) Deteriorated conjugate c) Deteriorated microwells due to improper storage d) Deteriorated substrate e)Contaminated tips may contaminate the reagent bottle	a) Repeat assay with a check on each step. Adhere to assay procedure detailed in package insert b) Check for turbidity, change of color or expiry and repeat the assay c) Check storage temperature (2-8°C) and conditions; Check for traces of moisture d) Check for change in color of substrate reagent e) Use always fresh tips while aspirating reagents from vials; while dispensing reagents do not touch the sides of the wells
Very high OD of positive control (>3.0)	Reader standardization to be checked	Most readers have linearity between 2.5 and 3.0. Check data on linearity of the instrument being used
Yellow tinge across entire plate	a) Over incubation with conjugate or substrate b) Substrate / Stop solution not fresh c) Plate left standing for too long before reading on the plate reader d) Substrate incubation carried out in light e) Incubation temperature is not maintained as specified in protocol f)Drying of wells during wash cycles	a) Keep a check on incubation time, Retest b) Check the reagents for change in color c) Read results within 5 minutes of adding the stop solution d) Use the dark cover provided during substrate incubation e) Antibodies have optimum binding activity at the specified temperature f) Always ensure washing solution to be present during wash cycles.
Inconsistent absorbance across the plate (poor reproducibi-lity)	a) Inconsistent pipetting b) Improper mixing of specimens and reagents c) Inconsistent washing	a) Keep a check on pipetting technique (volume aspirated and dispensed each time) b) Follow consistent and uniform technique

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