TRUSTwell®

MALARIA Ag ELISA Kit

REF

AE0611

IVD

- 96-well ELISA kit for the qualitative detection of Malaria Antigen in human blood samples.
- Store at 2-8 °C upon receipt

INTENDED USE

The TRUSTwell Malaria Ag ELISA Kit is a solid phase enzyme linked immunosorbent assay for the qualitative detection of malaria antigen in human blood sample. It is intended for professional use only as an aid in the diagnosis of infection with plasmodium species. Any reactive specimen with the TRUSTwell Malaria Ag ELISA Kit must be confirmed with alternative testing method(s) and clinical findings.

INTRODUCTION

Malaria is mosquito-borne infectious disease that affects humans and other animals[1]. Malaria causes symptoms that typically include fever, tiredness, vomiting and headache[2]. In severe cases, it can cause yellow skin, seizures, coma or death[2]. Symptoms usually begin ten to fifteen days after being bitten by an infected mosquito[3].

If not properly treated, people may have recurrences of the disease months later[3]. In those who have recently survived an infection, reinfection usually causes milder symptoms. The disease is widespread in the tropical and subtropical regions that exist in a broad band around the equator[2]. The disease now occurs in more than 90 countries worldwide, and it is estimated 229 million cases of malaria worldwide resulting in an estimated 2.7 million deaths per year[4]. Malaria is usually confirmed by the microscopic examination of blood films or by antigen-based rapid diagnostic tests.

TEST PRINCIPLE

The TRUSTwell Malaria Ag ELISA Kit is a solid phase enzyme linked immunosorbent assay based on the principle of the sandwich ELISA for the detection of malarial antigen in human whole blood sample.

The TRUSTwell Malaria Ag ELISA Kit is composed of two key components:

1) Solid microwells pre-coated with monoclonal antibodies;
2) Liquid conjugates composed of Anti-HRP II and Anti-pLDH conjugated with horse radish peroxidase (HRP- Anti-HRP II and Anti-pLDH conjugates).

During the assay, the test specimen is first incubated with the coated microwells. After the first incubation period, the unbounded protein matrix is removed with a subsequent wash step. In the second incubation with HRP-Anti-HRP II and Anti-pLDH conjugate is added after incubation and washing to remove all the unbounded enzyme conjugate. The presence of the complexed conjugates is shown by a blue color upon additional incubation with TMB Substrate. The reaction is stopped with Stop Solution and absorbances are read using a spectrophotometer at 450 /620-690 nm.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	Microwells coated with Anti-HRP II and Anti-pLDH monoclonal antibodies	8 wells x 12 strips	AE0611W
2	Positive control	0.75 mL	AE0611P
3	Negative control	0.75 mL	AE0611N
4	Sample Diluent	12 mL	AE0611SD
5	Enzyme conjugate (51 X concentrate)	0.3 mL	AE0611H
6	Conjugate Diluent	12 mL	AE0611CD
7	Wash buffer (30 X concentrate)	20 mL	AWE3000
8	TMB substrate (Ready to use)	12 mL	ATME2000S
9	Stop solution	12 mL	ASE1000
10	ELISA Working Sheet	2 Nos	PI-AE001E
11	Product insert	1 No.	PI-AE0611
12	Desiccant	4 Nos	
13	Sealant	3 Nos	

Materials and reagents required but not provided in the kit

1. Pipette capable of delivering 10 µL, 50 µL, and 100 µL volumes with a precision better than 1.5%.
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable.
3. Absorbent paper for blotting the microplate wells.
4. Parafilm or other adhesive film sealant for sealing plate.
5. Timer.
6. Distilled or de-ionized water.

STORAGE AND STABILITY

All reagents requiring refrigeration immediately after use. Reseal the microwells after removing the desired number of wells. Ensure that the reagents are brought to room temperature before opening. All the reagents are stable through the expiration date printed on the label if not opened. Do not freeze the kit or expose the kit over 8°C.

WARNING AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test.
2. Failure to follow the insert gives inaccurate test results.
3. Do not use expired devices.
4. Bring all reagents to room temperature (18°C-28°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood specimen for testing.
7. Do not ingest the reagents. Avoid contact with eyes, skin and mucose. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
9. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens. Dispose of all specimens and materials used to perform the test as biohazardous waste.
10. In the beginning of each incubation and after adding Stop Solution, gently rock the microwells to ensure thorough mixing. Avoid the formation of air bubbles as which results in inaccurate absorbance values. Avoid splash liquid while rocking or shaking the wells
11. Don't allow the microplate to dry between the end of the washing operation and the reagent distribution.
12. The enzyme reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or substrate solution.
13. The substrate solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. TMB Substrate must be stored in the dark.
14. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
15. The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.
16. Avoid strong light during color development.

MATERIALS AND REAGENTS

1. Whole blood specimen obtained by acceptable venipuncture technique.
2. This kit is designed for use with whole blood specimen without additives only.
3. If a specimen is not tested immediately, refrigerated at 2°C-8°C. If storage period greater than three days are anticipated, the specimen should be frozen (-20°C). Avoid repeated freezing-thawing of specimens. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
4. Specimens containing precipitants may give inconsistent test results.

SPECIMEN COLLECTION AND PREPARATION

1. Bring all reagents, controls to room temperature (18°C-28°C).
2. Dilute concentrated Wash Buffer 30-fold with water as following:

Plate	DI water	30 X wash buffer	Final volume
Full plate	580 mL	20 mL	600 mL
Half plate	290 mL	10 mL	300 mL
A quarter plate	145 mL	5 mL	150 mL

Warm up the concentrated Wash Buffer at 37˚C to dissolve the precipitant if it appears. Mix each reagent before adding to the test wells.

3. Dilute enzyme conjugate 51 times (for example add 20 µL of conjugate (51X) to 1 mL conjugate diluent).

No.of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
Conjugate (51 X) uL	20	40	60	80	100	120	140	160	180	200	220	240
Conjugate Diluent (mL)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0

4. Determine the number of microwells needed and mark on the ELISA Working Sheet with the appropriate information. Positive and Negative Controls require to be run in duplicate to ensure accuracy.

ASSAY PROCEDURE

1. Remove the desired number of strips and secure them in the microwell frame. Reseal un-used strips.
2. Add specimens according to the designation on the ELISA Working Sheet
   2.1. **Blank wells:** Leave the blank well alone (2 wells). Don't add any reagents (Optional).
   2.2 **Control wells:** Add 100 µL of Positive control (2 wells), Negative Control (2 wells), respectively.
   Test wells: Add 100 µL of Sample Diluent to all the test wells, then transfer 25 µL of each test specimen to each test well, respectively.

To ensure better precision, use pipette to handle solution.

3. Gently rock the plate wells for twenty seconds, then cover the plate with sealant.
4. Incubate the wells at 37°C for 60 minutes.

5. Carefully remove the incubation mixture by emptying the solution into a waste container. Fill each well with diluted wash buffer (350 µL per well) and shake gently for 20-30 seconds. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 5 more times.
6. Add 100 µL of HRP conjugates into each well except the blank well, cover the plate,
7. Incubate at 37°C for 30 minutes.
8. Wash the plate 6 times as step 5 described.
9. Add 100 µL of ready to use TMB substrate into each well including the blank well.
10. Incubate at 37˚C in dark for 20 minutes.
11. Stop the reaction by adding 100 µL of stop solution to each well. Gently mix for 20 seconds. It is important to make sure that all the blue color changes to yellow color completely.
12. Set the microplate reader wavelength at 450nm and measure the absorbance (OD) of each well against the blank well within 10 minutes after adding Stop Solution. A filter of 620-690nm can be used as a reference wavelength to optimize the assay result

Flow chart of assay procedure

1.	Secure strips in microwell frame	Number of strips
2.	Add controls(NC,PC) to designated wells	100 µL
3.	Add sample diluent	100 µL
4.	Add specimens Gently rock	25 µL
5.	Incubate	37°C, 60 minutes
6.	Wash: manual or automatic	6 times
7.	Add Conjugate Gently rock	100 µL 20 seconds
8.	Incubate	37°C, 30 minutes
9.	Wash: manual or automatic	6 times
10.	Add ready to use TMB substrate Gently rock	100 µL 20 seconds
11.	Incubate	37°C, 20 minutes
12.	Add Stop Solution. Gently mix	100 µL 20 seconds
13.	Read the result	450/620-690 nm within 10 minutes

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cut-off value = 0.12 + NC

(For Example: OD of N.C – 0.010)= 0.12 + 0.010 Cut -off Value = 0.130

B. Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the Cut-off value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cut - off Value}}$$

C. Assay validation

The mean OD value of the Malaria positive controls should be ≥ 0.50. The mean OD value of the Malaria negative controls should be ≤ 0.10.

If above specification is not met, the assay is Invalid. Check the assay procedure including incubation time and temperature and repeat assay.

D. Interpretation of the results

Specimen OD ratio

Negative < 1.00 Positive ≥ 1.00

1. The negative result indicates that there is no malaria antigen in the specimen.
2. Results just below the cut-off value (Lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to retest in duplicate the corresponding specimens when it is applicable).
3. Specimens with above the cut-off value considered to be positive by the TRUSTwell Malaria Ag ELISA Kit.
4. Specimens with equal to cut-off value are initially considered to be positive by the TRUSTwell Malaria Ag ELISA Kit. They should be retested in duplicate before final interpretation.
5. If after re-testing of a specimen, the absorbance value of the 2 duplicates are less than the cut-off value, the initial result is non repeatable and the specimen is considered to be negative with the TRUSTwell Malaria Ag ELISA Kit.

Non repeatable reactions are often caused by:

- Inadequate microwell washing,
- Contamination of the substrate solution by oxidizing agents (bleach,metal ions,etc.)
- Contamination of the stopping solution

If after retesting the absorbance of one of the duplicates is equal or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the TRUSTwell Malaria antigen ELISA Kit, subject to the limitation of the procedure, described below.

PERFORMANCECHARACTERISTICS

Clinical Performance

A total of 661 blood specimens were collected from a malaria-endemic area which were characterized by the microscopic smear test, the same samples were tested by TRUSTwell Malaria Ag ELISA Kit. A comparison for all specimens is shown in the following table:

	TRUSTwell Malaria Ag ELISA Kit		
Smear test	**Positive**	**Negative**	**Total**
Pf Positive	43	0	43
Pv Positive	148	1	149
Negative	1	468	469
Total	192	469	661

Relative Sensitivity:99.47 %, Relative Specificity:99.78%, Overall Agreement:99.68%

Analytical Sensitivity

During the internal evaluation of three different batches, the detection limit of pLDH was established at ≥ 10 parasites/µL while tested with the 1st WHO International Standard for Plasmodium vivax antigen (LDH), NIBSC Code: 19/116. Similarly, the detection limit of Pf HRP-II was established at ≥ 20 parasites/µL while tested with Positive Control Wells (PCWs) from Microcoat & FIND standards.

Precision

Intra-assay precision was determined by assaying 20 replicates of three negatives, three low positives and three high positives

Specimens	Number of specimens	No. of replicates	CV
Negatives	3	20	≤50%
High positives	3	20	≤10%
Low positives	3	20	≤10%

Inter-assay precision was determined by assaying 20 replicates of one negative, one low positive and one high positive

Specimens	Number of specimens	No. of replicates	CV
Negatives	1	20	≤50%
High positives	1	20	≤10%
Low positives	1	20	≤10%

Cross Reactivity

No cross-reactivity was observed while testing 10 positives specimens from each of the following disease states or special conditions, respectively:
Dengue Chikungunya HBsAg HIV Covid

Interference

Common substances (such as pain and fever medication and blood components) may affect the performance of the TRUSTwell Malaria Ag ELISA Kit. Interference was studied by spiking these substances into 3 clinical specimens: negative, Malaria antigen low positive and high positive. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the TRUSTwell Malaria Ag ELISA kit

List of potentially interfering substances and concentrations tested:

1. Salicylic acid 4.34 mmol/L
2. Sodium citrate 1.3 %
3. Creatinine 442 µmol/L
4. EDTA 3.4 µmol/L
5. Glucose 55 mmol/L
6. Heparin 3,000 U/L
7. Bilirubin 10 mg/dL

LIMITATION OF THE TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of Malaria antigen in whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The TRUSTwell Malaria Ag ELISA Kit is limited to the qualitative detection of Malaria antigen in whole blood.
3. A negative result for an individual subject indicates absence of detectable malaria antigen. However, a negative test result does not preclude the possibility of exposure to or infection with malaria.
4. A negative result can occur if the quantity of malaria antigen present in the specimen is below the detection limits of the assay.
5. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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4. WHO (2020). World Malaria Report 2020. Switzerland: World Health Organization. ISBN 978-92-4-001579-1.

Athenese-Dx Pvt. Ltd.
Module No. 407 & 408, 4th Floor,
TICEL Bio Park II, No. 5, CSIR Road.
Taramani, Chennai-600113, India
Tel: +91-44-22541131
E-mail: info@athenesedx.com
Website: www.athenesedx.com

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Index of Symbols

	See instructions for use
IVD	For *in vitro* diagnostic use only
REF	Catalog #
LOT	Lot Number
	Use by
Σ N	Tests per kit
2°C 8°C	Store between 2-8 °C
	Do not reuse
	Manufacturer
	Date of manufacture