TRUSTwell®

HBsAg ELISA Kit

REF

AE0710

IVD

- 96-well ELISA kit for the qualitative detection of HBsAg in human serum or plasma
- Store at 2-8°C upon receipt

INTENDED USE

The TRUSTwell HBsAg ELISA Kit is a solid phase enzyme linked immunosorbent assay for the qualitative detection of hepatitis B virus surface antigen (HBsAg) in human serum or plasma. It is intended for professional use only as an aid in the diagnosis of infection with HBV. Any reactive specimen with the TRUSTwell HBsAg ELISA Kit must be confirmed with alternative testing method(s) and clinical findings.

INTRODUCTION

Hepatitis virus B (HBV) is the most common cause of persistent viremia and the most important cause of chronic liver disease and hepatocellular carcinoma. Clinically apparent HBV infections may have been extant for several millennia. It is estimated that there are 300 million chronic carriers of HBV in the world. The carrier rates vary from as little as 0.3% (Western countries) to 20% (Asia, Africa)[1].

HBV is a hepatotropic DNA virus. The core of the virus contains a DNA polymerase[2], the core antigen (HBcAg)[3] and the e antigen (HBeAg)[4]. The core of HBV is enclosed in a coat that contains lipid, protein and carbohydrate and expresses an antigen terms hepatitis B surface antigen (HBsAg)[3].

HBsAg is the first marker to appear in the blood in acute hepatitis B, being detected 1 week to 2 months after exposure and 2 weeks to 2 months before the onset of symptoms. Three weeks after the onset of acute hepatitis almost half of the patients will still be positive for HBsAg. In the chronic carrier state, the HBsAg persists for long periods (6-12 months) with no seroconversion to the corresponding antibodies. Therefore, screening for HBsAg is highly desirable for all donors, pregnant women and people in high-risk groups.

TEST PRINCIPLE

TRUSTwell HBsAg ELISA Kit is a solid phase enzyme linked immunosorbent assay based on the principle of antibody sandwich technique for the detection of HBsAg in human serum or plasma.

The TRUSTwell HBsAg ELISA Kit is composed of two key components:

1) Solid microwells pre-coated with monoclonal anti HBsAg antibody;

2) Liquid conjugates composed of polyclonal anti-HBsAg conjugated with horse radish peroxidase (HRP-HBsAb conjugates).

During the assay, the test specimen and HRP-HBsAb conjugates are incubated simultaneously with the coated microwells. HBsAg, if present in the specimen, reacts to the anti-HBsAg antibody coated on the microwell surface as well as the HRP-HBsAb conjugate, forming sandwich complex conjugates.

Unbounded conjugates are then removed by washing. 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 absorbance 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-HBsAg Ab	8 wells x 12 strips	AE0710W
2	HBsAg negative control	1 mL	AE0710N
3	HBsAg positive control	1 mL	AE0710P
4	Enzyme Conjugate	6 mL	AE0710H
5	Wash Buffer (30X concentrate)	20 mL	AWE3000
6	TMB substrate A	6 mL	ATME2000A
7	TMB substrate B	6 mL	ATME2000B
8	Stop solution	12 mL	ASE1000
9	ELISA Working Sheet	2 Nos	AE0001ES
10	Product insert	1 No.	PI-AE0710

Materials and reagents required but not provided in the kit

1. Pipette capable of delivering 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 450 nm wavelength is acceptable.
3. Absorbent paper for blotting the microplate wells.
4. Distilled or De-ionized water.
5. Parafilm or other adhesive film sealant for sealing plate.
6. Timer.
7. Incubator.

STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Return 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. Failure to follow the insert gives inaccurate test results.
2. Do not use expired devices.
3. Bring all reagents to room temperature (18°C-28°C) before use.
4. Do not use the components in any other type of test kit as a substitute for the components in this kit.
5. Do not use hemolized blood specimen for testing.
6. 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.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Dispose of all specimens and materials used to perform the test as biohazardous waste.
10. In the beginning of each incubation and after adding Stopping Solution, gently rocking 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. The Substrate B 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.

SPECIMEN COLLECTION AND PREPARATION

1. Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
2. This kit is designed for use with serum or plasma 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. Clarify such specimens by centrifugation prior to assaying.
5. Do not use serum specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PREPARATION OF THE REAGENTS

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	290 mL	10 mL	300 mL
Half plate	145 mL	5 mL	150 mL
A quarter plate	72.5 mL	2.5 mL	75 mL

Warm up the concentrated Wash Buffer at 37 °C to dissolve the precipitant if it appears.

4. Mix each reagent before adding to the test wells.
5. 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 wells alone (2 wells). Don't add any reagents.
   - 2.2 Control wells: Add 50 µL of HBsAg Positive Control (2 wells), Negative Control (2 wells) into the designated control wells, respectively.
   - 2.3 Test wells: Add 50 µL of test specimens into each test well, respectively.

   To ensure better precision, use pipette to handle solution.
3. Add 50 µL of the HRP- HBsAb conjugates to each well, except the blank well.

4. Gently rock the wells for twenty second, then cover the wells.
5. Incubate the wells at 37 °C for to 90 minutes.
6. Carefully remove the incubation mixture by empting 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 4 more times.
7. Add 50 µL of TMB substrate A and 50 µL of TMB substrate B into each well including the blank well.
8. Incubate at 37 °C in dark for 20 minutes.
9. Stop the reaction by adding 100 µL of stop solution to each well. Gently mix for 20-30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
10. Set the microplate reader wavelength at 450 nm and measure the absorbance (OD) of each well against the blank well within 15 minutes after adding Stop Solution. A filter of 620–690 nm 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 or specimens	50 µL
3.	Add conjugate Gently rock	50 µL 20 seconds
4.	Incubate	37°C, 90 minutes
5.	Wash: manual or automatic	5 times 350 µL/well
6.	Add TMB substrate A and B Gently rock	50 µL + 50 µL 20 seconds
7.	Incubate	37°C, 20 minutes
8.	Add Stop Solution. Gently rock	100 µL 20 seconds
9.	Read result	450/620-690 nm within 15 minutes

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cutoff value = **0.13 + NC**

NC: Mean OD value of Negative Control.

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 Blank should be ≤ 0.08

The mean OD value of the HBsAg positive controls should be ≥ 0.50.

The mean OD value of the HBsAg negative controls should be ≤ 0.10.

If above specification are 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 detectable HBsAg 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 cut-off ≥ 1.00 are initially considered to be positive by the TRUSTwell HBsAg ELISA Kit. They should be retested in duplicate before final interpretation.

   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 HBsAg ELISA Kit.

   Non repeatable reactions are often caused by:
   - Inadequate microwell washing,
   - Contamination of negative specimens by serum or plasma with a high antibody titer,
   - Contamination of the substrate solution by oxidizing agents (bleach, metal ions, etc.)
   - Contamination of the stopping solution

   If after re-testing 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 HBsAg ELISA Kit, subject to the limitation of the procedure, described below.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

Analytical sensitivity of the assay has been estimated at 0.1 ng/ml for both HBsAg Ad and HBsAg Ay, the results is presented as the minimum detection limit when the standards spiked into 20 different negative specimens.

Clinical Performance

A total of 1033 patient specimens from susceptible subjects were tested by the TRUSTwell HBsAg ELISA Kit. Comparison for all subjects is showed in the following table:

	TRUSTwell HBsAg ELISA Kit		
Ref. HBsAg EIA	**Positive**	**Negative**	**Total**
Positive	105	0	105
Negative	2	926	928
Total	107	926	1033

Relative Sensitivity:100%,Relative Specificity:99.78%,Overall Agreement:99.81%

Precision

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

Specimens	Number of specimens	Number of replicates	CV
Negatives	3	20	5.6 – 20 %
High positives	3	20	3.0 -5.9 %
Low positives	3	20	6.4 -11.4 %

Cross reactivity

No false positive HBsAg ELISA test results were observed on 10 positives specimens from each of the following disease states or special conditions, respectively:

HIV HCV Syphilis Dengue Malaria Typhoid

Interference

Common substances (such as pain and fever medication and blood components) may affect the performance of the TRUSTwell HBsAg ELISA Kit. Interference was studied by spiking these substances into 3 HBsAg clinical specimens: negative, low positive and high positive. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the TRUSTwell HBsAg 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 HBsAg in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The TRUSTwell HBsAg ELISA Kit is limited to the qualitative detection of HBsAg at a sensitivity level of 0. 1 ng/mL in human serum or plasma. The intensity of color does not have linear correlation with the antigen titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable HBsAg. However, a negative test result does not preclude the possibility of exposure to or infection with HBV.
4. A negative result can occur if the quantity of HBsAg present in the specimen is below the detection limits of the assay (below 0.1 ng/mL), or the HBsAg that are detected are not present during the stage of disease in which a specimen is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. 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. Magnius LO, Espmark A. A new antigen complex co-occurring with Australia antigen. Acta Pathol Microbiol Scand [B] Microbiol Immunol. 1972;80(2):335-7

Athenese-Dx Pvt. Ltd.

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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
	Tests per kit
	Store between 2-8 °C
	Do not reuse
	Manufacturer
	Date of manufacture