fortress diagnostics

ISO 13485 accredited company

BXE0742A

96 Tests

STORE AT 2-8°C

FOR IN- VITRO DIAGNOSTIC USE ONLY

HBsAg (HS)

High sensitivity - ELISA

Fortress HBsAg is an in vitro diagnostic kit for the detection of hepatitis B surface antigen (HBsAg) in human serum or plasma.

Intended Use:

1. For screening of blood donors.
2. For monitoring individuals with a higher than normal risk of contracting hepatitis, e. g. patients, technicians or nursing personnel in renal dialysis units or clinical laboratories
3. As an aid in the diagnosis of liver disease

Principle of the Assay:

The test is an enzyme-immunoassay based on a 'sandwich' principle. Polystyrene microtiter strip wells have been coated with monoclonal anti-HBs(antibody to HBsAg). Patients serum or plasma sample is added to the microwells. During incubation, the specific immune-complex formed in case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum proteins, second antibody conjugated to the enzyme HRP and directed against a different epitope of HBsAg is added to the wells. During the second incubation step, these HRP conjugated antibodies will be bound to any anti-HBs-HBsAg complexes previously formed during the first incubation, and the unbound HRP conjugate is then removed by washing. After washing to remove unbound HRP conjugate, chromogen solutions containing TMB and Urea peroxidise are added to the wells. In presence of the antibody-antigen-antibody HRP sandwich immune-complex, the colourless chromogens are hydrolyzed by the bound HRP conjugate to a blue coloured product. The blue colour turns yellow after stopping the reaction using the Stop solution. The colour intensity can be measured and it is proportional to the amount of antigen captured in the wells and to its amount in the sample respectively. Wells containing samples negative for HBsAg remain colourless.

Assay principle scheme: Double antibody sandwich ELISA

Ab(p)+Ag(s)+(Ab)ENZ →[Ab(p)–Ag(s)–(Ab)ENZ]→blue→yellow (+)

Ab(p) +(Ab)ENZ → [Ab(p)]→ no color (-)

Incubation I	Inc II	Immobilized Complex	Colouring	Results
60 min.	30 min		30min.	

Ab(p)–pre-coated anti–HBs antibodies;

Ag(s)–HBsAg antigens in sample;

(Ab)ENZ–HRP conjugated anti-HBs;

Kit Contents: Store at 2-8°C

HbsAg Kit Contents;	Volume
Microwell Plate 96 Tests	1 plate (12x8/8x12 well strips per plate)
Negative Control	1x1ml
Positive Control	1x1ml
HRP – Conjugate Reagent	1x6ml
HbsAg Sample Diluent	1x5ml
Stock Wash Buffer	1x30ml (Dilute 1 to 20 with distilled water before use. Once diluted, stable for two weeks at 2-8°C).
Chromogen Solution A	1x6ml (Ready to use and once open, stable for one month at 2-8°C)
Chromogen Solution B	1x6ml (Ready to use and once open, stable for one month at 2-8°C)
Stop Solution	1x6ml
Plastic Sealable Bag	1 Unit
Plate Cover	1 Sheet
Package Inserts	1 Copy

Additional Materials And Instruments Required But Not Provided:

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Disposable V-shaped troughs.
5. Dispensing system and/or pipette (single or multichannel),disposable pipette tips
6. Absorbent tissue or clean towel.
7. Dry incubator or water bath, 37±0.5°C.
8. Microplate shaker for dissolving and mixing conjugate with samples.
9. Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
10. Microwell aspiration/wash system.

Specimen Collection and Transportation:

1. Sample Collection:

Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or haemolysed samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.

2. Transportation and Storage:

Store samples at 2-8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower).Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labelled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

Special Instructions for Washing Plates:

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles with dispensing of 350-400µl/well, are sufficient to avoid false positive reactions and high background (all wells turn yellow).
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells, but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer's liquid dispensing channels are not blocked or contaminated, and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to perform at least 5cycles, dispensing 350-400µl/well and aspirating the liquid for 5times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution(final concentration of 2.5%) for 24 hours, before liquids are disposed in an appropriate way.
7. The concentrated Washing solution should be diluted **1 to 20** before use. For one plate, mix 30 ml of the concentrate with 570ml of water for a final volume of 600ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

Storage and Stability:

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8 °C, **do not freeze**. To assure maximum performance of this HBsAg ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

Precautions and Safety:

Fortress HbsAg ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature(18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37° inside the incubator.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV ½, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121°C or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (2M H_2SO_4) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of such substances.
20. Materials Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

Assay Procedure:

Step1 Reagents preparation:

Allow the reagents and samples to reach room temperature (18-30°C) for at least 15-30minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the stock Wash Buffer **1 to 20** with

distilled or deionized water. Use only clean vessels to dilute the buffer.

Step2 Numbering Wells:
Set the strips needed in strip-holder and sufficient number of wells including three Negative controls(e.gB1,C1,D1), two Positive Control(e.g.E1,F1) and one Blank(e.g>E1,F1) and one Blank (e.g.A1, neither samples nor HRP Conjugate should be added into the blank well).If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

Step3 Adding Sample Diluent:
Add 20ul of Sample Diluent to each well except the Blank, and mix by taping the plate gently.

Step4 Adding Sample:
Add 100ul of Positive control, negative control and specimen into their respective wells. Note: Use a separate disposable tip for each specimen, Negative control and Positive control to avoid cross contamination.

Step5 Incubation:
Cover the plate with the plate cover and incubate for **60 minutes at 37°C**. It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

Step6 Adding HRP Conjugate:
Add 50ul HRP Conjugate to each well except the Blank and mix by tapping the plate gently.

Step7 Incubation II:
Cover the plate with the plate cover and incubate for 30 minutes for 37°C as in Step 5.

Step8 Washing:
At the end of the incubation, remove and discard the plate cover. Wash each well **5** times with diluted Wash buffer. Each time, allow the microwells to soak for 30-60seconds. After the final washing cycle, turn the plate down onto blotting paper or clean towel, and tap it to remove any remainders.

Step9 Colouring:
Dispense **50µl** of Chromogen A and **50µl** Chromogen B solution into each well including the **Blank**, and mix by tapping the plate gently. Incubate the plate at **37°C for 15minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue colour in Positive control and HBsAg positive sample wells.

Step10 Stopping Reaction:
Using a multichannel pipette or manually, add **50µl** Stop Solution into each well and mix gently. Intensive yellow colour develops in Positive control and HBsAg positive sample wells.

Step11 Measuring the Absorbance:
Calibrate the plate reader with the blank well and read the absorbance at **450nm**.If a dual filter instrument is used, set the reference wavelength at **630nm**. Calculate the Cut-off value and evaluate the results (**Note:** read the absorbance within **5** minutes after stopping the reaction).

Interpretation of Results:
Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample's optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

1. Calculation of Cut-off value

Cut-off value (C.O.) = *Nc × 2.1

*Nc = the mean absorbance value for three negative controls.
Important: If the mean OD value of the negative control is lower than 0.05, take it as 0.05 .If higher than 0.05 see the Quality control range.

Exaample:
1.Calculation of No:

Well No	B1	C1	D1
Negative conttrols DD value	0.02	0.012	0.016

Nc= 0.016 (the Nc Value is lower than 0.05 so take it as(0.05
Calculation of Cut-off value: Cut off(C.O)=0.05x2.1=0.105

If one of the Negative control values does not meet the Quality control range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

2. Quality control range:
The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed
1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
2. .The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm, or at 450nm after blanking.
3. .The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.

3. Interpretations of the results:
(S = the individual absorbance (OD) of each specimen)
Negative Results (S/C.O. <1): samples giving an absorbance less than the Cut-off value are considered negative, which indicates that no hepatitis B surface antigen has been detected with this HBsAg ELISA kit, therefore the patient is probably not infected with hepatitis B virus.
Positive Results (S/C.O.≥1): samples giving an absorbance greater than or equal to the Cut-off value are considered initially reactive, which indicates that HBV surfaces antigen has probably been detected with this HBsAg ELISA kit. Any initially reactive samples should be retested in duplicates. Repeatedly reactive samples could be considered positive for HBsAg , therefore the patient is probably infected by HBV and the blood unit should not be transfused.
Borderline: Samples with absorbance to Cut-off ratio between 0.9 and 1.00 are considered borderline samples and retesting is recommended. Repeatedly positive samples can be considered positive for HBsAg.

Fortress HBsAG 3rd Gen Performance:

Clinical Specificity: The clinical specificity of this assay was determined by a panel of samples obtained from 4476 healthy blood donors and 6344 hospitalized patients.

	Sample	-	+	Specificity
Donors	4476	4471	5	99.89%
Patients	6344	6340	4	99.94%

Clinical Sensitivity: 1) A panel of 40 serum samples including 26 positive confirmed samples.

PANEL	BACKGROUND	FORTRESS HBSAG 3RD GEN	
		+	-
CDC	+	26	0
	-	0	14
DETECTION RATE		100 %	

2. A panel of 108 samples sequenced by PCR method.

BACKGROUND		NUMBER	FORTRESS HBSAG 3RD GEN
adr(+)	Wildtype	35	33
	4 mutations	5	4
adw(+)	Wild type	37	34
	16 mutations	25	24
ayw(+)	Wild type	2	2
	2 mutations	2	2
ayr(+)	2 mutations	2	2
TOTAL		108	101

3. Two seroconversion panels from BBI

CODE	DAYS	FORTRESS HBSAG 3RD GEN
PHM909	0	0.03
	4	0.07
	7	0.16
	9	1.64
	14	5.09
	18	17.17
	21	27.10
PHM920	0	0.04
	5	0.03
	26	2.46
	35	27.62
	37	29.00
	42	28.40

Analytical Specificity:
1. No cross reactivity observed with samples from patients infected with HAV, HCV, HIV, CMV, and TP.
2. No interference from rheumatoid factors up to 2000U/ml observed.
3. No high dose hook effect up to HBsAg concentrations of 200000ng/ml observed during clinical testing.
4. Frozen specimens have been tested too to check for interferences due to collection and storage.

Analytical Sensitivity (lower detection limit):The sensitivity of the assay has been calculated by a panel of series of dilutions of WHO reference standard. The assay shows that lower detection limit reaches 0.1 IU/ml.

CONCENTRATION LEVEL	FORTRESS HBSAG 3RD GEN
0.5 IU/ml	+
0.2 IU/ml	+
0.1 IU/ml	+
0.05 IU/ml	+/-
0.025 IU/ml	-

Limitations:
1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of ELISA assays. The test is design to achieve very high performance characteristics of sensitivity and specificity. However, in very rare cases some HBV mutants or subtypes can remain undetectable. Antigens may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. If, after retesting of the initially reactive samples, the assay results are negative , these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
3 .Any positive results must be interpreted in conjunction with patient clinical information and other laboratory testing results.
4. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
5. The prevalence of the marker will affect the assay's predictive values.
6. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
7. This is a qualitative assay and the results cannot be use to measure antigens concentrations.

Indications of Instability or Deterioration of the Reagents:
1. Values of the Positive or Negative controls, which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones.
2. If after mixing of the Chromogen A and B solutions into the wells, the, the colour of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

Reference:

1 .Stevens, C. E., P. E. Taylor, and M. J. Tong. 1988. Viral hepatitis and liver disease. Alan R. Riss, New York, N.Y. 142. Stevens, C. E., P. E. Taylor, M. J. Tong, P. T. Toy, G. N. Vyas, P. V. Nair,
2. J.Y. Weissman, and S. Krugman. 1987. Yeast-recombinant hepatitis B vaccine. Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. JAMA 257:2612–2616. 143. Stevens, C. E., P. T. Toy, P. E. Taylor, T. Lee, and H. Y. Yip. 1992. Prospects for control of hepatitis B virus infection: implications of childhood vaccination and long term protection. Pediatrics 90(Suppl.):170–173.
3 .Hurie, M. B., E. E. Mast, and J. P. Davis. 1992. Horizontal transmission of hepatitis B virus infection to U.S. born children of Hmong refugees. Pediatrics 89:269–273.