AccuDiag™ HBsAg ELISA

Cat# 1701-12

BLOOD VIRUS SCREENING
HEPATITIS B VIRUS SURFACE ANTIGEN ELISA

Test	HBsAg ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Double Antibody Sandwich Principle
Detection Range	Qualitative: Positive & Negative Control
Sample	50ul
Specificity	99.75%
Sensitivity	99.9%
Total Time	~ 75 min
Shelf Life	12-14 Months from the manufacturing date

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

INTENDED USE

The purpose of the HBsAg ELISA Test is to diagnose patients who are suspected of having a hepatitis B virus infection and/or for blood donor screening. This HBsAg test is an enzyme-linked immunosorbent assay for the qualitative identification of HBsAg in human serum/plasma and one of the best methods available for either screening blood donors or in the clinical diagnosis of hepatitis B-infected individuals.

SUMMARY AND EXPLANATION

Hepatitis B infection is spread through infected blood or body secretions. Because Hepatitis B virus (HBV) is known as one of the major causes of blood transmitted hepatitis infections, blood screening using the HBsAg ELISA test is one of the most effective ways of preventing the spread of HBV. When using this test, It is important to classify hepatitis B infection through three phases of the infection - incubation, acute, and convalescent. This can be done by identifying serological markers through each phase. Knowing the serological markers of HBsAg is an excellent method for the diagnosis and treatment of infected individuals. Symptoms of Hepatitis B virus (HBV) infection can range from mild to severe, including chronic liver disease (cirrhosis and carcinoma). Hepatitis B virus is an enveloped, double-stranded DNA virus belonging to the Hepadnaviridae family. The outer envelope surface antigen of the Hepatitis B virus is HBsAg. It contains the determinant "a" and is identified in two subgroups (ay and ad). HBV has four HBsAg subtypes (adw, ady, ayw, and ayr) and has 10 major serotypes. HBsAg can be identified two to four weeks before the ALT levels are abnormal, and three to five weeks before symptoms appear.

TEST PRINCIPLE

The HBsAg ELISA Test kit employs an antibody sandwich ELISA technique where monoclonal antibodies unique to HBsAg, are pre-coated on polystyrene microwell strips. The serum or plasma sample is added together with a second antibody, the HRP Conjugate, (horseradish peroxidase) and directed against a different epitope of HBsAg. Throughout the time of incubation, specific immunocomplex that may have formed (indicating presence of HBsAg) is captured on the solid phase. After washing, to eliminate serum proteins and unbound HRP-conjugate, chromogen solutions containing tetramethyl-benzidine (TMB) and urea peroxide are added to the wells. Next, the colorless chromogens are hydrolyzed by the bound HRP-conjugate to a blue-colored product while in the presence of the antibody-antigen-antibody (HRP) sandwich immunocomplex. Halting the reaction with sulfuric acid, the blue color then turns yellow. The color intensity can be gauged proportionally to the amount of antigen captured in the wells, and to the amount in the sample, respectively. The wells remain colorless if the HBsAg result is negative.

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	Immobilized Complex	Coloring	Results
60		**15 min.**	

Ab(p)-pre-coated anti-HBs antibodies;
Ag(s)-HBsAg antigens in sample;
(Ab)ENZ-HRP conjugated anti-HBs;

SPECIMEN COLLECTION AND PREPARATION

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 lipemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause deterioration of the target proteins in sample.
2. **Transportation and Storage**: Store samples at 2-8ºC.Samples not required for assaying within 3 days should be stored frozen (-20ºC or lower).Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

SPECIAL INSTRUCTIONS FOR WASHING

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. It is recommended that the washing system should be calibrated 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 buffer 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.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Microwell Plate**
   Blank microwell strips fixed on white strip holder.
   The plate is sealed in aluminum pouch with desiccant.
   8×12-well strips per plate.
   Each well contains monoclonal antibodies reactive to HBsAg (anti-HBs).
   The microwell strips can be broken to be used separately. Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return to 2~8°C.
2. **Negative Control**
   Yellowish liquid filled in a vial with green screw cap.
   1ml per vial.
   Protein-stabilized buffer tested non-reactive for HBsAg.
   Preservatives: 0.1% ProClin 300.
   Ready to use as supplied.
   Once open, stable for one month at 2-8°C.
3. **Positive Control**
   Red-colored liquid filled in a vial with red screw cap.
   1ml per vial.
   HBsAg diluted in protein-stabilized buffer.
   Preservatives: 0.1% ProClin 300.
   Ready to use as supplied. Once open, stable for one month at 2-8°C.
4. **HRP-Conjugate Reagent**
   Red-colored liquid filled in a white vial with red screw cap.
   7ml per vial.
   Horseradish peroxidase-conjugated anti-HBs antibodies.
   Ready to use as supplied. Once open, stable for one month at 2-8°C.
5. **Wash Buffer**
   Colorless liquid filled in a clear bottle with white screw cap.
   30ml per bottle.
   PH 7.4, 20 × PBS (Contains Tween-20 as a detergent).
   DILUTE BEFORE USE -The concentration must be diluted **1 to 20** with distilled/deionized water before use. Once diluted, stable for one week at room temperature or for two weeks at 2-8°C.
6. **Chromogen Solution A**
   Colorless liquid filled in a white vial with green screw cap.
   8ml per vial.
   Urea peroxide solution.
   Ready to use as supplied.
   Once open, stable for one month at 2-8°C
7. **Chromogen Solution B**
   Colorless liquid filled in a black vial with black screw cap.
   8 ml per vial.
   TMB solution (Tetramethyl-benzidine dissolved in citric acid).
   Ready to use as supplied.
   Once open, stable for one month at 2-8°C.
8. **Stop Solution**
   Colorless liquid filled in a white vial with yellow screw cap.
   8 ml per vial.
   Diluted sulfuric acid solution (2.0M H_2SO_4)
9. **Plastic Sealable Bag**
   For enclosing the strips not in use.
10. **Cardboard Plate Cover**
    To cover the plates during incubation and prevent evaporation or contamination of the wells.
11. **Package Insert**

Materials 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. Microshaker 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.

ASSAY PROCEDURE

1. **Reagents preparation:** Allow the reagents and samples to reach room temperature (18-30ºC) 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 37º until crystals dissolve. Dilute the stock Wash Buffer 1 to 20 with distilled or deionized water. Use only clean vessels to dilute the buffer.
2. **Numbering Wells:** Set the strips needed in strip-holder. and number sufficient number of wells including three for the Negative control **(e.g. B1, C1, D1)**, two for the Positive control **(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.
3. **Adding Sample and HRP-Conjugate:** Add 50µl of Positive control, Negative control, and specimen into their respective wells. **Note: Use a separate disposal pipette tip for each specimen, Negative and Positive Control as to avoid cross-contamination.** Add 50µl **HRP-Conjugate** to each well except the Blank, and mix by tapping the plate gently.
4. **Incubating:** Cover the plate with the plate cover and incubate for **60 minutes at 37ºC**. 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.
5. **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.
6. **Coloring:** 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 **37º for 15minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and HBsAg positive sample wells.
7. **Stopping Reaction:** Using a multichannel pipette or manually, add 50µl Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and HBsAg positive sample wells.
8. **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).

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.

Example:
1. Calculation of Nc:

Well No	B1	C1	D1
Negative controls OD value	0.02	0.012	0.016

Nc= 0.016 (the Nc value is lower than 0.05 so take it as 0.05)
2. Calculation of Cut-off value: Cut-off (C.O) = 0.05 x 2.1 = 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 optical density (OD) of each specimen)

Negative Results (S/C.O. <1): samples giving an optical density less than the Cut-off value are considered negative, which indicates that no hepatitis B virus surface antigen has been detected with this HBsAg ELISA, therefore the patient is probably not infected with hepatitis B virus.

Positive Results (S/C.O.≥1): samples giving an optical density 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 . Any initially reactive samples should be retested in duplicates. Repeatedly reactive samples could be considered positive for HBsAg, therefore the patient is probably infected with 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.

PERFORMANCE CHARACTERISTICS

Clinical Specificity: The clinical specificity of this assay was determinate by a panel of samples obtained from 2500 healthy blood donors and 300 undiagnosed hospitalized patients. The repeatedly reactive samples and samples confirmed positive with the reference test were not included in the calculation of specificity.

	Sample	-	+	Confirmed positive	Specificity	False positive
donors	2500	2447	56	53	99.87%	3
patients	300	274	27	26	99.63%	1

Clinical Sensitivity: The clinical sensitivity of this HBsAg ELISA was calculated by a panel of samples obtained from 670 hepatitis B patients with well-characterized clinical history based upon reference assays for detection of HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc. Licensed HBsAg ELISA test was used as a confirmatory assay. The evaluation results are given below. Results obtained in individual laboratories may differ.

	Sample	-	+	Confirmed positive	Sensitivity	False Negatives
Acute	200	0	200	200	100%	0
Chronic	400	0	399	400	99.75%	1
Recovery	70	65	5	5	100%	0

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 Endpoint Sensitivity (lower detection limit): The assay shows sensitivity at the Cut-off of 0.5 ng/ml (adr) and 0.5 ng/ml (adw, ay).

Reproducibility:		Within Run		Between Run	
Specimen Type	**No**	**Mean OD**	**CV%**	**Mean OD**	**CV%**
5ng/ml HBsAg	**10**	**0.175**	**10.6%**	**0.150**	**11.0%**
Weak positive	**10**	**0.457**	**9.0%**	**0.432**	**9.5%**
Moderate positive	**10**	**1.572**	**7.0%**	**1.437**	**7.5%**
Strong positive	**10**	**2.327**	**4.2%**	**2.302**	**4.4%**
Positive control	**10**	**2.322**	**4.1%**	**2.315**	**4.2%**

LIMITATIONS OF PROCEDURE

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 can not be used 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 color of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

VALIDITY

Please do not use this kit beyond the expiry date indicated on the kit box and reagent labels.

PRECAUTIONS

This kit is intended **FOR IN VITRO USE ONLY**

FOR PROFESSIONAL USE ONLY

The 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 (18-30º) before use. Shake reagent gently before, and return to 2-8º 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 37º 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 121ºC 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, alkalins 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.

STORAGE

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

ISO 13485
ISO 9001

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