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IVD | See external label | 2°C 8°C | Σ 96 tests | REF 1702-12

AccuDiag™ HBsAb ELISA

Cat # 1702-12

(Qualitative)
ANTIBODIES TO HEPATITIS B VIRUS CORE ANTIGEN ELISA

Test	HBsAb ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Sandwich ELISA: Double Antibody
Detection Range	Qualitative Positive; Negative control & Cut off
Sample	50ul Serum
Specificity	99.70%
Sensitivity	100%
Total Time	~ 75 min
Shelf Life	12 -18 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 **HBsAb ELISA Test** is for clinical lab diagnosis of patients who are suspected of having a hepatitis B virus infection. This anti-HBs ELISA test is an enzyme-linked immunosorbent assay for in vitro qualitative identification of antibodies to hepatitis B surface antigen (anti-HBs) in human serum/plasma.

SUMMARY

The effects of HBV infection range anywhere from mild to severe hepatitis which includes chronic liver problems, such as carcinoma and cirrhosis. As part of the Hepadnaviridae family, HBV is an enveloped, double-stranded DNA virus that is a primary cause of hepatitis transmission through blood. In order to classify hepatitis B infection, the serological markers need to be identified during the three phases of the infection - incubation, acute, and convalescent.

An important protein of the envelope structure of the virus is the hepatitis B surface antigen (HBSAg) which appears soon after infection. During the acute phase of the disease, HBsAg is detectable in blood and this is what makes it a key serological marker for identification and diagnosis of HBV. There is recovery after treatment. There can also be a progression to long chronic carrier stage if the infection has progressed for more than six months. It is at the acute critical stage of infection that a strong immunological response occurs. Markers for recovery are the expanding titers of HBsAg neutralizing antibodies (anti-HBs). Crucial reasons why serological detection of anti-HBs is important: a critical technique for follow up with HBV infected patients, monitoring of their vaccination with synthetic and natural HBsAg-based vaccines, and possible prevalence studies of HBV.

PRINCIPLE OF THE ASSSAY

The HBsAb ELISA Test kit employs an antigen sandwich ELISA technique where polystyrene microwell strips are pre-coated with recombinant HBsAg. The test begins by having a combination of patient's serum or plasma sample being added to Horseradish Perioxidase (HRP-Conjugate) to the microwells. If anti-HBs is present, the pre-coated and conjugated antigens will be bound to the two variable domains of the antibody. A specific immunocomplex is formed and captured on the solid phase during incubation. When washing is complete (to remove unbound HRP-conjugates and sample serum proteins), chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells. The colorless chromogens are hydrolyzed by the bound HRP-Conjugate to a blue-colored product when there is a presence of antigen-antibody-antigen (HRP) sandwich complex. After stopping the reaction with sulfuric acid, the blue color turns yellow. The color intensity can be gauged proportionally to the amount of antibody captured in the wells, and to the amount in the sample, respectively. The wells remain colorless if the anti-HBs result is negative.

Assay principle scheme: Double antigen sandwich ELISA

Ag(p) +Ab(s) + (Ag)ENZ → [Ag(p)–Ab(s)–(Ag)ENZ] →Blue→Yellow (+)

Ag(p) + (Ag)ENZ → [Ag (p)] →No Color (-)

Incubation	Immobilized Complex	Coloring	Results
60 min.		15min.	

Ag(p)–pre-coated HBsAg;

Ab(s)– anti-HBs in sample;

(Ag)ENZ–HRP conjugated HBsAg;

COMPONENTS

96 Tests

- **MICROWELL PLATE** 1 plate
  Blank microwell strips fixed on a white strip holder.
  The plate is sealed in aluminium pouch with desiccant.
  8×12/12×8-well strips per plate. Each well contains purified HBsAg. 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.
- **NEGATIVE CONTROL** 1vial
  Yellowish liquid filled in a vial with green screw cap.
  1ml per vial. Protein-stabilized buffer tested non-reactive for anti-HBs.
  Preservatives: 0.1% ProClin 300.
  Ready to use as supplied. Once open, stable for one month at 2-8°C.
- **POSITIVE CONTROL** 1vial
  Red-colored liquid filled in a vial with red screw cap.
  1ml per vial. anti-HBs 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.
- **HRP-CONJUGATE REAGENT** 1vial
  Red-colored liquid filled in a white vial with red screw cap.
  6.5ml per vial. Horseradish peroxidase-conjugated HBsAg.
  Ready to use as supplied. Once open, stable for one month at 2-8°C.
- **STOCK WASH BUFFER** 1 bottle
  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 concentrate 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 when stored at 2-8°C.
- **CHROMOGEN SOLUTION A** 1 vial
  Colorless liquid filled in a white vial with green screw cap.
  7ml per vial. Urea peroxide solution.

Ready to use as supplied.
Once open, stable for one month at 2-8 ℃.

- **CHROMOGEN SOLUTION** B — 1 vial
  Colorless liquid filled in a Black vial with Black screw cap.
  7 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 ℃.
- **STOP SOLUTION** — 1vial
  Colorless liquid filled in a white vial with white screw cap.
  7ml per vial.Diluted sulfuric acid solution (2.0 M H_2SO_4).
  Ready to use as supplied.
- **PLASTIC SEALABLE BAG** — 1unit
  For enclosing the strips not in use.
- **CARBOARD PLATE COVER** — 1sheet
  To cover the plates during incubation and prevent evaporation or contamination of the wells.
- **PACKAGE INSERTS** — 1 copy

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED BUT NOT PROVIDED

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

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

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 hemolized 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℃. Samples not required for a ssaying within 3 days should be stored frozen (-20℃ 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.

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

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

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 (18-30°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°C 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, 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.

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 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.

Step3 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.

Step4 Incubating: 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.

Step5 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 30-60 seconds. After the final washing cycle, turn the plate down onto blotting paper or clean towel, and tap it to remove any remainders.

Step6 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 **37°C 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.

Step7 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.

Step8 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 AND QUALITY CONTROL

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: <u>Cut-off value (C.O.) = *Nc × 2.1</u>**

***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 negative, for this assay which indicates that no hepatitis B virus surface antigen have probably not been detected with this kit. Therefore, there are no serological indications for past infection and the individual is not immune against infection with HBV.

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 antibodies to HBV surfaces antigen have been detected using this anti-HBs ELISA kit. Retesting in duplicates of any reactive samples is recommended. Repeatedly reactive samples could be considered positive for anti-HBs. Elevated concentration of anti-HBs are indication for recovery and immunity to HBV.

Borderline (S/C.O. =0.9-1.1): Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these samples in duplicates is recommended to confirm the results. Repeatedly positive samples can be considered positive for antibodies to HBsAg.

Follow-up and supplementary testing of any positive samples with other HBV assays is required before establishing of the final diagnosis.

TEST PERFORMANCE AND EXPECTEC RESULTS

Analytical Endpoint Sensitivity (lower detection limits): The assay shows sensitivity near the Cut-off of 5mIU/ml.

Clinical Specificity: The clinical specificity of the assay has been determinate by a panel of samples obtained from 1500 healthy blood donors and 250 undiagnosed hospitalized patients.

Clinical Sensitivity: The clinical sensitivity of the assay has been calculated by a panel of samples obtained from 580 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 anti-HBs ELISA test was applied as a confirmatory assay. For establishing the test performance characteristics during monitoring of post-vaccination antibody response, additional group of samples from 200 individuals receiving HBV vaccine was tested for anti-HBs.

The evaluation results are given below. Results obtained in individual laboratories may differ.

Specificity	**Samples**			**True positive**	**Specificity**	**False positive**
	No.	-	+			
Donors	1500	869	631	630	99.88%	1
Patients	250	140	110	109	99.29%	1
TOTAL	1750	1011	741	739	99.58	2

Sensitivity	**Samples**			**True positive**	**Sensitivity**	**False negatives**
	No.	-	+			
Acute	350	345	5	5	100%	0
Chronic	130	130	0	0	100%	0
Recovery	100	5	95	95	100%	0
Vaccine recipients	200	7	193	193	100%	0

TOTAL	780	486	293	293	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. The assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein.
4. No high dose hook effect up to 150000mIU/ml.
5. Frozen specimens have been tested too to check for interferences due to collection and storage.

Reproducibility:		**Within Run**		**Between Run**	
Specimen Type	No	Mean OD	CV%	Mean OD	CV%
Weak positive	10	0.457	9.0%	0.443	9.5%
Moderate positive	10	1.570	7.5%	1.497	7.0%
Strong positive	10	2.310	4.4%	2.250	4.2%
30mIU	10	0.523	6.2%	0.508	6.7%
Positive control	10	2.132	4.2%	2.016	4.3%

LIMITATIONS

1. Non-repeatable positive result may occur due to the general biological of the ELISA assays. This assay is designed to achieve very high performance characteristics of sensitivity and specific and the ‘’sandwich model’’ minimizes the unspecific reactions due to interference with unknown matters in sample. However, in very rare cases some HBV mutants or subtypes can remain undetectable. A negative results with an antibody detection test does not preclude the possibility of infection.
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. Any positive results must be interpreted in conjunction with patient clinical information and other laboratory results.
3. Common source 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, samples nature and quality.
4. The prevalence of the marker will affect the assay’s predictive values.
5. Samples tested using test kits from different manufacturer can give similar qualitative results but some samples can give discrepancies due to the antibodies diversity and the antigenic properties of HBsAg used in the assay.
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 antibodies 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 rage, 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 solution into the wells, the, the color of the mixture turns blue within few 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.

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SUMMARY OF THE ASSAY PROCEDURE:	
Add Sample	**50µl**
Add HRP-Conjugate	**50µl**
Incubate	**60minutes**
Wash	**5times**
Coloring	**50µl A + 50µl B**
Incubate	**15minutes**
Stop the reaction	**50µl stop solution**
Read the absorbance	**450nm or 450/630 nm**

Date Adopted	Reference No.
2008-01-05	DA-HBsAb-2009

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