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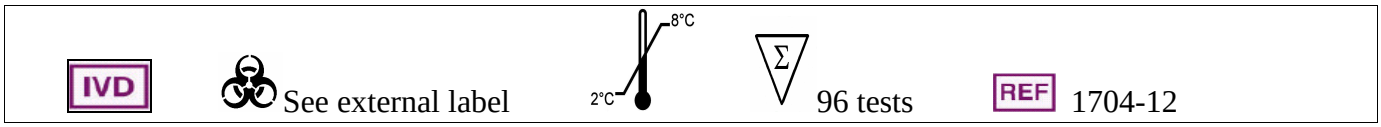

AccuDiag™ HBcAb ELISA

Cat # 1704-12

ANTIBODIES TO HEPATITIS B VIRUS CORE ANTIGEN ELISA Kit

Test	**HBcAb ELISA**
Method	**Enzyme Linked Immunosorbent Assay**
Principle	**Competitive ELISA**
Detection Range	**Qualitative Positive; Negative control & Cut off**
Sample	**50ul Serum**
Specificity	**99.82%**
Sensitivity	**99.92%**
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 intended use for this HBcAb ELISA test is for clinical lab diagnosis and handling of hepatitis B-infected patients. The HBcAb ELISA is an enzyme-linked immunosorbent assay for the qualitative identification of antibodies to hepatitis B antigen (HBcAg) in human serum/plasma.

SUMMARY

As part of the Hepadnaviridae family, HBV is an enveloped, double-stranded DNA virus that is a primary cause of hepatitis transmission through blood. The effects of HBV infection range anywhere from mild to severe hepatitis, which includes chronic liver problems, such as carcinoma and cirrhosis. 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. The main component of the virus is Hepatitis B core antigen (HBcAg). This core antigen is comprised of a single polypeptide of approximately 17kD that is discharged upon disaggregation of the core particles. At least one immunological determinant is present in the antigen.

Shortly after the onset of HBsAg, antibodies to HBcAg (anti-HBc total antibody and IgM) appear and never go away. In isolated cases, a Hepatitis B infection can be contracted without immunologically detectable anti-HBc. This is found usually in immunosuppressed patients. Screening for anti-HBc yields data on the prevalence of hepatitis B in various populations. This is because anti-HBc is a marker of acute, chronic, or resolved HBV infection. The identification of anti-HBc is vital when being diagnosed in a clinical setting. Together with other hepatitis B tests, the anti-HBc marker allows correct diagnosis and proper monitoring of progress of the virus. Anti-HBc may possibly be the only indicator of a hepatitis B infection (including other tests of HBsAg-negative patients).

PRINCIPLE OF THE ASSAY

The system of the HBcAb ELISA test is founded on the solid phase, one-step incubation competitive principle. When anti-HBc is present, it competes with monoclonal anti-HBc conjugated to horseradish peroxidase (HRP-Conjugate) for a fixed amount of purified HBcAg pre-coated in the wells. If no anti-HBc is present, HRP-conjugated anti-HBc will be bound together with antigens inside the wells. In the course of washing, any unbound HRP-Conjugate is removed. After chromogen solutions A and B are added into the wells and during incubation, a blue-colored product appears when the colorless chromogens are hydrolyzed by the bound HRP-Conjugate. After the reaction is stopped with sulfuric acid, the blue color turns yellow. A presence of antibodies to HBcAg in the sample is indicated by low color, or no color present at all.

Assay principle scheme: Competition ELISA

Ag(p)+Ab(s)+(Ab)ENZ → [Ag(p)–Ab(s)] → No color (+)
Ag(p) +(Ab)ENZ → [Ag(p) – (Ab)ENZ] → Blue →Yellow Color (-)

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

Ag(p)–pre-coated HBcAg;
Ab(s)– anti-HBc in sample;
(Ab)ENZ– HRP conjugated anti-HBc;

COMPONENTS


96 Tests

- **MICROWELL PLATE** 1plate
  Blank microwell strips fixed on a white strip holder.

The plate is sealed in aluminum pouch with desiccant.
8×12/12×8-well strips per plate. Each well
contains purified HBcAg. 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℃.

- **NEGATIVE CONTROL** 1vial
  Yellowish liquid filled in a vial with green screw cap.
  1 ml per vial.
  Protein-stabilized buffer tested non-reactive for anti-HBc.
  Preservatives: 0.1% ProClin 300.
  Ready to use as supplied.
  Once open, stable for one month at 2-8℃.
- **POSITIVE CONTROL** 1vial
  Red-colored liquid filled in a vial with red screw cap.
  1 ml per vial.
  Purified anti-HBc diluted in protein stabilized buffer
  containing preservatives: 0.1% ProClin 300.
  Ready to use as supplied.
  Once open, stable for one month at 2-8℃.
- **HRP-CONJUGATE REAGENT** 1vial
  Red-colored liquid filled in a white vial with red / orange screw cap.
  6.5 ml per vial.
  Horseradish peroxidase-conjugated anti-HBc.
  Ready to use as supplied.
  Once open, stable for one month at 2-8℃.
- **STOCK WASH BUFFER** 1bottle
  DILUTE BEFORE USE
  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).
  The concentrate must be diluted 1 to 20 with distilled
  or deionized water before use. Once
  diluted, stable for one week at room temperature or for
  two weeks when stored at 2-8℃.
- **CHROMOGEN SOLUTION A** 1vial
  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** 1vial
  Colorless liquid filled in a Brown vial with brown / black screw cap.
  7ml 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.
- **STOP SOLUTION** 1vial
  Colorless liquid filled in a white vial with yellow / white screw cap.
  7ml per bottle.
  Diluted sulfuric acid solution (2.0M H_2SO_4).
  Ready to use as supplied.

- **PLASTIC SEALABLE BAG** 1unit
  For enclosing the strips not in use.
- **CARDBOARD PLATE COVER** 1sheet
  To cover the plates during incubation, and prevent the well from evaporation or contamination.
- **PACKAGE INSERTS** 1copy

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℃.
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.

SPECIMEN COLLECTION, TRANSPORTAION 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 inactivated 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.
3. **Sample Preparation**: Each sample must be diluted 1:30 with normal saline.

SPECIAL INSTRUCTIONS FOR WAHSING

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℃; do not freeze. To assure maximum performance of this anti-HBc ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

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℃) before use. Shake reagent gently be fore, and return to 2-8℃ 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.
8. Avoid the formation of air-bubbles when adding the reagents. 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℃ 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 ºC 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 Negative controls **(e.g. B1, C1, D1)** two Positive controls **(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** of HRP-Conjugate to each well except into the Blank and mix by tapping the plate gently.

Step4 Incubating: Cover the plate with the plate cover and incubate for **60minutes 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 **5times** with diluted Wash buffer. Each time, allow the microwells to soak for 30-60seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remaining liquids.

Step6 Coloring: Dispense **50µl** of Chromogen A and **50µl** Chromogen B solution into each well including the **Blank**. Incubate the plate at **37℃ for 15minutes, avoiding light** . The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate will produce blue color in Negative control and anti-HBc negative 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 Negative control and anti-HBc negative 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 5minutes 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 (C.O.) = *Nc × 0.5
***Nc** = the mean absorbance value for three negative controls.

Example: of Cut-off calculation:
1. Calculation
Well No: B1 C1 D1
Negative controls OD value 1.720 1.715 1.717
Nc=1.717
2. Calculation of Cut-off (C.O.)=1.729 x 0.5=0.858

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 450nm.
2. The OD value of the Negative control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.
3. The OD value of the Positive 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 greater than the Cut-off value are considered negative, which indicates that no antibodies to HBV core antigen have been detected using this anti-HBc ELISA kit. This result should not be used alone to establish the infection state.
Positive Results (S/C.O. ≤1): Samples giving absorbance less than, or equal to the Cut-off value are initially reactive for this assay, which indicates that antibodies to HBV core antigen have probably been detected with this anti-HBc ELISA kit. Any initially reactive samples should be retested in duplicates. Repeatedly reactive samples could be considered positive for anti-HBc. A positive result with anti-HBc detection is an indication of acute HBV infection. Determination of anti-HBc is useful method for screening of blood donors and in serological monitoring during follow-up of chronic HBV carriers. However, any positive result should not be used alone to establish the infection state.
Borderline (S/CO =0.9-1.1): Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline samples and retesting is recommended. Repeatedly reactive samples could be considered positive for anti-HBc.

TEST PERFORMANCE AND EXPECTED RESULTS

The **clinical specificity** of this assay has been determinate by a panel of samples obtained from 1683 healthy blood donors and 145 undiagnosed hospitalized patients. The repeatedly reactive samples and samples confirmed positive with the reference test were not included in the calculation of the specificity.

The **clinical sensitivity** of this anti-HBc ELISA kit have been calculated by a panel of samples obtained from 975 hepatitis B patients with well-characterized clinical history based upon reference assays for

detection of HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc. This panel included samples from acute, chronic and recovered hepatitis B patients. Licensed anti-HBc ELISA test was used as a confirmatory assay. The evaluation results are given below. Results obtained in individual laboratories may differ.

Specificity	Samples	-	+	Confirmed positive	Specificity	False Positive
Blood donors	1683	566	1117	1115	99.64%	2
Patients	145	80	65	65	100%	0
TOTAL	1828	646	1182	1180	99.82	2

Sensitivity	Samples	-	+	Confirmed positive	Sensitivity	False Negative
Acute	429	11	417	418	99.76%	1
Chronic	105	0	105	105	100%	0
Recovery	441	5	436	436	100%	0
TOTAL	975	16	958	959	99.92	1

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 during clinical testing.
3. The assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein.
4. Frozen specimens have been tested to check for interferences due to collection and storage.

Reproducibility	No runs	Within run Mean OD	Within run CV%	Between run Mean OD	Between run CV%
Weak positive	10	0.639	5.8%	0.645	6.4%
Moderate positive	10	0.394	7.4%	0.404	8.0%
Strong positive	10	0.012	21%	0.017	22%
Negative control	10	1.768	4.5%	1.702	4.6%

LIMITATIONS

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of the ELISA method. The test is designed to achieve very high performance characteristics of sensitivity and specificity. However, in very rare cases some HBV mutants or subtypes can remain undetectable. Antibodies may be also undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. Any positive results must be interpreted in conjunction with patient clinical information and other

laboratory testing results.

3. 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.
4. The prevalence of the marker will affect the assay's predictive values.
5. This is a qualitative assay and the results cannot be use to measure antibodies concentrations.
6. 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.

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 expiration indicated on the kit box and reagent labels.

SUMMARY OF THE ASSAY PROCEDURE:	
Dilute samples	1:30
Add Sample	100 µl
Add controls	50 µl
Add HRP-Conjugate	50 µl
Incubate	60 minutes
Wash	5 times
Coloring	50µl A + 50µl B
Incubate	15 minutes
Stop the reaction	50µl stop solution
Read the absorbance	450 or 450/630 nm

Date Adopted	Reference No.
2008-01-05	DA-AccuDiag™ HBcAb ELISA-2009

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