Hepatitis B Virus Diagnostics

Anti-HBc ELISA (Quantitative)
Diagnostic Kit for Quantitative Determination of Antibody to Hepatitis B Virus Core Antigen (Sandwich ELISA)

Read the package insert carefully and completely before performing the assay. Follow the instructions and do not modify them. Only by strict adherence to these instructions, the erroneous results can be avoided and the optimal performance of this anti-HBc ELISA (Quantitative) achieved.

INTENDED USE
This anti-HBc ELISA (Quantitative) is an enzyme linked immunosorbent assay (ELISA) for in vitro quantitative determination of antibodies to hepatitis B virus core antigen (anti-HBc) in human serum or plasma. It is intended for use in clinical laboratories for diagnosis and management of patients related to infection with hepatitis B virus.

SUMMARY
Hepatitis B virus (HBV) is an enveloped, double-stranded DNA virus belonging to the hepadnavirus family and is recognized as the major cause of blood transmitted hepatitis together with hepatitis C virus (HCV). Infection with HBV induces a spectrum of clinical manifestations ranging from mild, asymptomatic disease to fulminant hepatitis, severe chronic liver diseases, which in some cases can lead to cirrhosis and carcinoma of the liver. Classification of a hepatitis B infection requires the identification of a number of serological markers expressed during infection and screening for anti-HBc provides with information on the disease. In the absence of other hepatitis B markers (HBsAg, anti-HBc), anti-HBc may be the only indication of an existing hepatitis B viral infection.

PRINCIPLE OF THE TEST
For detection of anti-HBc, this kit uses antigen "sandwich" ELISA method where polystyrene microwell strips are pre-coated with purified HBcAg. Patient’s serum or plasma sample is added to the microwells together with a second HBcAg conjugated to Horseradish Peroxidase (HRP-Conjugate). In case of presence of anti-HBc in the sample, the pre-coated and conjugated antigens will be bound to the two variable domains of the antibody and during incubation, the specific immune complex formed is captured on the solid phase. After washing to remove sample and unbound HRP-Conjugates, Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells. In presence of the antigen-antibody-antigen(HRP) "sandwich" complex, the colorless Chromogens are hydrolyzed by the bound HRP-Conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively.

In Vitro Diagnostic Use Only
This kit contains reagents sufficient for testing of maximum of 90 specimens in a test run.

STANDARDS
(6x0.5ml per vial Vial) preserv.0.1% ProClin™ 300

CALIBRATION CURVE STANDARDS: Yellowish liquid filled in a vial with screw cap.

This kit contains the following standards: 0IU/ml, 0.1IU/ml, 0.3IU/ml, 0.6IU/ml, 1.2IU/ml and 2.0IU/ml.

Anti-HBc diluted in protein-stabilized buffer.

Ready to use as supplied. Once open, stable for 4 weeks at 2-8°C.

Ag | HRP

Code 1 (1x12ml per vial)
preserv.0.1% ProClin™ 300

HRP-CONJUGATE: Red-colored liquid in a white vial with red screw cap.

HORSEHADISH PEROXIDASE-CONJUGATED HBcAG.

Ready to use as supplied. Once open, stable for 4 weeks at 2-8°C.

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE
1. Specimen Collection: No special patient’s preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum or plasma specimens can be used with 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 to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the specimen should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, but highly lipaemic, icteric, or hemolytic specimens should not be used as they can give false results in the assay. Do not heat inactivate specimens. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.

3. This anti-HBc ELISA (Quantitative) is intended ONLY for testing of individual serum or plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

4. Transportation and Storage: Store specimens at 2-8°C. Specimens not required for assaying within 1 week should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, specimens should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

5. Reagents preparation: Allow the reagents to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.

6. Use only sufficient volume of sample as indicated in the procedure steps and do not modify them.

7. Do not exchange reagents from different lots or use reagents from incorrect result, strictly follow the test procedure steps and do not modify them.

8. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so may cause low sensitivity of the test.

9. Assure that the incubation temperature is 37°C inside the incubator.

10. When adding specimens, do not touch the well’s bottom with the pipette tip.

11. If using fully automated equipment, 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.

12. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.

13. When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.

14. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.

15. When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.

16. The enzymatic activity of the HRP-conjugate might be affected from individual serum or plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

17. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date.

18. 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 anti-HBc ELISA (Quantitative), during storage, protect the reagents from contamination with microorganism or chemicals.

19. CAUTION - CRITICAL STEP: Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.

20. 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.

21. Avoid assay steps long time interruptions. Assure same working conditions for all wells.

22. Indicators of instability/ deterioration of the reagent:

   Values of the Positive or Negative controls, which are out of the indicated quality control range, are indicators 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 resultand substantial deterioration or instability of the reagents, immediately replace the reagent with new one.

23. PROCEDURE

   Reagents preparation: Allow the reagents to reach room temperature (18-30°C). Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and only clean vessels to dilute the buffer. All other reagents are READY TO USE AS SUPPLIED.

   Step1 Preparation: Mark six calibration curve standards wells (e.g. B1-G1) 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. Run the standards in duplicates.

   Step4 Preparating of the standards in duplicates.

   Step2 Adding Diluent: Add 50μl of Specimen Diluent into each well except the Blank.

   Step3 Addin Sample: Add 50μl of Calibration curve standards and Specimen into their respective wells except the Blank.

   Step5 Incubating: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.

   Step6 Adding HRP-Conjugate: Add 100μl of HRP-Conjugate into each well except the Blank. Mix by tapping the plate gently.

   Step7 Incubating: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.

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

   Step9 Coloring: Add 50μl of Chromogen A and 50μl of Chromogen B solutions into each well including the Blank.

   Step10 Stopping Reaction: Using a multichannel pipette or manually, add 50μl of Stop Solution into each well and mix gently. The blue color will turn yellow after stopping the reaction.

   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 filter at 450nm or at 450/630nm.
used, set the reference wavelength at 630nm. Calculate the value and evaluate the results. (Note: read the absorbance within 10 minutes after stopping the reaction).

**INSTRUCTIONS FOR WASHING**

1. A good washing procedure is essential in order 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 of 350-400μl/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with specimen or HRP-conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. If the microplate washer 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 carry out 5 washing cycles, dispensing 350-400μl/well and aspirating the liquid for 5 times. 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 at a final concentration of 2.5% for 24 hours, before they are wasted in an appropriate way.
7. The concentrated Wash buffer should be diluted 1 to 20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

**QUALITY CONTROL AND CALCULATION OF THE RESULTS**

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. If the result reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD from the print report values of specimens and standards. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of specimens and standards.

1. Record the absorbance (OD) obtained from the print report of the microplate reader.
2. Plot the absorbance for each calibration standard on the Y (LN-OD) solution.
3. Draw the standard curve through the plotted points (best-fit).
4. To determine the concentration of anti-HBc for an unknown, locate the absorbance (OD) value obtained from the print report of the unknown on the X-axis of the graph. Calculate the concentration of the unknown in IU/ml.

Example of a Standard Curve:
For illustration purpose only, the average values are given only: (*0IU/ml=Negative Sample or Negative Control)

<table>
<thead>
<tr>
<th>Standards</th>
<th>OD of Values of Standards</th>
<th>LN-OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1IU/ml</td>
<td>-2.303</td>
<td>-1.000</td>
</tr>
<tr>
<td>0.3IU/ml</td>
<td>-1.204</td>
<td>-0.511</td>
</tr>
<tr>
<td>0.6IU/ml</td>
<td>-0.511</td>
<td>-0.000</td>
</tr>
<tr>
<td>1.2IU/ml</td>
<td>0.182</td>
<td>0.392</td>
</tr>
<tr>
<td>2.0IU/ml</td>
<td>0.693</td>
<td>1.480</td>
</tr>
</tbody>
</table>

The linear regression equation derived is: Y=1.0710X-0.3344

If the OD value of one specimen is A=1.203, then its LN value is 0.185. substitute it into the equation, the anti-HBc concentration calculated is: EXP((0.185+0.3344)-1.0710) = 1.624 (IU/ml). (Note: The standard curve is for illustrative purpose only.)

Quality control (assay validation): The test results are valid if the Quality Control criteria are fulfilled. 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. If any following result is obtained, the test results should be considered invalid, it is necessary to repeat the test:
- r<0.99 after curve fitting by using the concentrations of the standards and its corresponding OD value
- The OD value of 2.0IU/ml standard <0.8
- The OD value of 0IU/ml standard >0.1

The abnormal points instandard curve may cause the deviation in the whole experimental results. Therefore, it is recommended to use double wells for each Standard in order to improve the accuracy of the assay. When only one Standard significantly gave a higher or lower value, and this is caused by human error, then this point can be discarded, the standard curve can be plotted with other Standards.

The valid range of linearity of this kit is 0.1-2.0IU/ml. The anti-HBc concentration in sample is beyond this range, it cannot obtain accurate quantitative value. If the concentration of sample is higher than 2.0IU/ml, the sample should be retested after diluted with Specimen Diluent.

The normal reference range of anti-HBc is <0.1IU/ml. Because of the difference in region, race, gender and age, it is suggested that laboratories establish their own reference range.

**PERFORMANCE CHARACTERISTICS**

Within the valid range of 0.1-2.0IU/ml, the linear correlation coefficient r should be not less than 0.9900.

Repeatability: the variation coefficient should not be higher than 15.0%.

**LIMITATIONS**

1. Non-repeatable reactive results may be obtained with any ELISA test due to the general characteristics of this method. Any positive result must be interpreted in conjunction with the patient clinical information and other laboratory results.
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. Calibration Curve Standards must be added for each test run, and the results must be obtained from the standard curve each time, otherwise it may lead to a large deviation in quantitative 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. Samples tested using assay from different manufacturer can give similar quantitative results but some samples can give discrepancies due to the antibodies diversity and the antigenic properties of HBcAg used in the assay.
7. 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.

**REFERENCES**


**SUMMARY OF THE MAJOR COMPONENTS OF THE KIT**

Use this summary only as a reference and always follow the comprehensive method sheet when performing the assay. Note: the components of individual kits are not lot-interchangeable.

<table>
<thead>
<tr>
<th>Microwell plate</th>
<th>Code 5</th>
<th>one</th>
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<tbody>
<tr>
<td>Calibration Curve Standards</td>
<td>Code 6</td>
<td>6x0.5mL</td>
</tr>
<tr>
<td>HRP-Conjugate</td>
<td>Code 9</td>
<td>1x12mL</td>
</tr>
<tr>
<td>Specimen Diluent</td>
<td>Code 9</td>
<td>1x12mL</td>
</tr>
<tr>
<td>Wash Buffer</td>
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<td>Code 2</td>
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<tr>
<td>Chromogen Solution B</td>
<td>Code 3</td>
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<tr>
<td>Stop Solution</td>
<td>Code 4</td>
<td>1x6mL</td>
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**SUMMARY OF THE ASSAY PROCEDURE**

Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

<table>
<thead>
<tr>
<th>Step</th>
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<tbody>
<tr>
<td>Add Specimen Diluent</td>
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<tr>
<td>Add Samples/Calibration Curve Standards</td>
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<tr>
<td>Incubate</td>
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<tr>
<td>Wash</td>
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<td>Add HPR-Conjugate</td>
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<td>Incubate</td>
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<td>Wash</td>
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<tr>
<td>Coloring</td>
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<tr>
<td>Incubate</td>
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<td>Stop the reaction</td>
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<td>Read the absorbance</td>
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**EXAMPLE SCHEME OF CONTROLS / SAMPLES DISPENSING**

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