Hepatitis B Virus Diagnostics

Anti-HBs ELISA (Quantitative)

Diagnostic Kit for Quantitative Determination of Antibody to Hepatitis B Virus Surface Antigen (ELISA)

**INTENDED USE**

This anti-HBs ELISA (Quantitative) is an enzyme linked immunosorbent assay (ELISA) for in vitro quantitative determination of antibodies to hepatitis B virus surface antigen (anti-HBs) in human serum or plasma for clinical purposes and assessing antibody response levels to HBsAg vaccine.

**SUMMARY**

Hepatitis B virus (HBV) is an enveloped, double-stranded DNA virus belonging to the Hepadnaviridae 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, inapparent disease to fulminant hepatitis, severe chronic liver diseases, with some cases can lead to cirrhosis and carcinoma of the liver. Classification of a hepatitis B infection requires the identification of several serological markers expressed during three phases (incubation, acute and convalescent) of the infection. Now the identification of several serological markers expressed during three phases of clinical samples and ethiological agents.

For detection of anti-HBs, this kit uses antigen "sandwich" ELISA method where polystyrene microwell strips are pre-coated with recombinant HBsAg. Patient's serum or plasma sample is added to the microwells together with a second HBsAg conjugated to Horseradish Peroxidase (HRP-Conjugate). In case of presence of anti-HBs 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 immunocomplex 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 84 specimens in a test run.

**PRINCIPLE OF THE TEST**

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**READ THE INSTRUCTIONS CAREFULLY AND COMPLETELY BEFORE PERFORMING THE ASSAY**

**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 hemolysis of the RBC.
2. 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.
3. Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, but highly lipemic, 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.
4. This anti-HBs 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.
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**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Freshly distilled or deionized water, disposable gloves and timer.
2. Appropriate waste containers for potentially contaminated materials, dispensing system and/or pipette, disposable pipette tips, absorbent tissue or clean towel, dry incubator or water bath, 37±1°C, plate reader, single wavelength 450nm or dual wavelength 450/630nm, microwell aspiration/wash system.

**CHROMOGEN SOLUTION A:** Colorless liquid filled in a whitevial. Diluted sulfuric acid solution (0.5M H2SO4).

**CHROMOGEN SOLUTION B:** Colorless liquid filled in a black vial with blackscREW cap. TMB (Tetramethylbenzidine) solution.

**STOP SOLUTION:** Colorless liquid in a white vial with whitescrew cap. Diluted sulfuric acid solution (0.5M H2SO4).

**PLASTIC SEALABLE BAG:** For enclosing the strips not in use 1unit

**PACKAGE INSERT**

2 sheets

**CARDBOARD PLATE COVER**

2 sheets

To cover the plates during incubation and prevent evaporation or contamination of the wells.

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

PRECAUTIONS AND SAFETY

TO BE USED ONLY FROM QUALIFIED PROFESSIONALS

The ELISA assays are kit and temperature sensitive. 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 for optimal performance of the tests.
2. 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 stated on labels or boxes.
3. 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.
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 the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
6. 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.
7. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
8. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Use different disposal pipette tips for samples/reagents dispensing. Use only number of strips required for 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. When measuring with a plate reader, determine the absorbance at 450nm or at 490nm.
12. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, carcinogenic effect as raw materials. Contact with the skin and the mucous should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.

PROCEDURE

Reagents preparation: Allow the reagents to reach room temperature (18-30°C). Check the Wash buffer concentration 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; H1-E2) 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.

Step2 Adding Sample: Add 50μl of Calibration curve standards and Specimen into their respective wells except the Blank. Use a separate disposal pipette tip for each specimen and standard to avoid cross-contamination.

Step3 Adding HRP-Conjugate: Add 50μl of HRP-Conjugate into each well except the Blank. Mix by tapping the plate gently.

Step4 Incubating: Cover the plate with the plate cover and incubate for 15 minutes at 37°C.

Step5 Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final wash cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.

Step6 Coloring: Add 50μl of Chromogen A and 50μl of Chromogen B solutions into each well including the Blank. Incubate the plate at 37°C for 15 minutes avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Calibration curve standards wells (except for 0μl/m/μl)and anti-HBs positive sample wells.

Step7 Stopping Reaction: Using a multichannel pipette or manually, add 50μl Stop Solution into each well and mix gently. The blue color will turn yellow after stopping the reaction.

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 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. Ensure that 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 avoid 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 10% sodium hypochlorite for 30 minutes to kill any microorganisms that may have been present. Also collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved.Materials Safety Data Sheet (MSDS) available upon request.
7. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucous should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.

8. 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 (log-OD) for each duplicate calibration standard on the Y (logarithmic ordinate) versus the corresponding anti-HBs concentration (log-mIU/ml) on the X (logarithmic abscissa) on double-logarithmic paper (do not average the duplicates of the calibration standards before plotting).

3. Draw the standard curve through the plotted points (best-fit).

4. To determine the concentration of anti-HBs for an unknown, locate the absorbance (OD) for each unknown on the Y-axis of the graph, find the intersecting point on the standard curve, and read the concentration (log-mIU/ml) from the X-axis of the graph. Calculate the concentration of the unknown in mIU/ml.

Example of a Standard Curve:
For illustration purpose only, the average values are given only:

<table>
<thead>
<tr>
<th>Standards</th>
<th>Log mIU/ml</th>
<th>Mean OD</th>
<th>log OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mIU/ml</td>
<td>1</td>
<td>0.186</td>
<td>-0.728</td>
</tr>
<tr>
<td>20mIU/ml</td>
<td>1.30103</td>
<td>0.380</td>
<td>-0.4202</td>
</tr>
<tr>
<td>40mIU/ml</td>
<td>1.62206</td>
<td>0.770</td>
<td>-0.1135</td>
</tr>
<tr>
<td>80mIU/ml</td>
<td>1.90309</td>
<td>1.427</td>
<td>0.1544</td>
</tr>
<tr>
<td>160mIU/ml</td>
<td>2.20412</td>
<td>2.249</td>
<td>0.3521</td>
</tr>
</tbody>
</table>

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.

1. The OD value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450 nm.
2. The OD value of 0mIU/ml standard must be < 0.100 at 450/630nm or at 450nm after blanking.
3. The OD value of 160mIU/ml standard must be > 1.500 at 450/630nm or at 450nm after blanking.

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 are due to the competitive nature of the test, and are common, most of which are related but not limited to inadequate washing step.

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 value.

5. In some cases, very strong immunological response after vaccination can be observed due to the vaccine biological characteristics. High concentrations of antibodies beyond the standard curve measurement range (> 160mIU/ml) can be diluted and retested. Samples may not show linear properties after dilution as the same as the materials used for the standards. This phenomenon is frequently observed when samples are tested for antibodies.

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 HBsAg 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


5. Engvall E. and Perlmann P. J.Immunochemistry, 8, 871-874, 1971


SUMMARY OF THE MAJOR COMPONENTS OF THE KIT

<table>
<thead>
<tr>
<th>Microwell plate</th>
<th>Code 5</th>
<th>one</th>
</tr>
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<tbody>
<tr>
<td>Calibration Curve Standards Code 6</td>
<td>6x0.5mL</td>
<td></td>
</tr>
<tr>
<td>HRP-Conjugate Code 6</td>
<td>1x6.5mL</td>
<td></td>
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<tr>
<td>Wash Buffer Code 1</td>
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<tr>
<td>Chromogen Solution A Code 2</td>
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<td></td>
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<tr>
<td>Chromogen Solution B Code 3</td>
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<td></td>
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<tr>
<td>Stop Solution Code 4</td>
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</tbody>
</table>

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.
SUMMARY OF THE ASSAY PROCEDURE

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

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>Add Samples/Calibration Curve Standards</strong></td>
<td>50μL</td>
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<tr>
<td><strong>Add HPR-Conjugate</strong></td>
<td>50μL</td>
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<tr>
<td><strong>Incubate</strong></td>
<td>60minutes</td>
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<tr>
<td><strong>Wash</strong></td>
<td>5times</td>
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<tr>
<td><strong>Coloring</strong></td>
<td>50μL A + 50μL B</td>
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<tr>
<td><strong>Incubate</strong></td>
<td>15minutes</td>
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<tr>
<td><strong>Stop the reaction</strong></td>
<td>50μL stop solution</td>
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<tr>
<td><strong>Read the absorbance</strong></td>
<td>450nm or 450/630 nm</td>
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EXAMPLE SCHEME OF CONTROLS / SAMPLES DISPENCING

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