HBcAb-IgM ELISA
Diagnostic Kit for IgM Antibodies to Hepatitis B Virus Core Antigen (ELISA)

INTENDED USE
HBcAb-IgM ELISA is an enzyme-linked immunosorbent assay (ELISA) for qualitative determination of IgM class antibodies to hepatitis B virus core antigen 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 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, which in some cases 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 three phases (incubation, acute and convalescent) of the infection. In chronic hepatitis, however, spikes of anti-HBc IgM synthesis are present, confirming reactivation of HBV in hepatocytes and giving origin to permanent IgM low titers. Presence of IgM and total anti-HBc indicates an ongoing or recent HBV infection. When used in conjunction with tests for other HBV serological markers, a laboratory diagnosis or a rule out of HBV infection can be achieved.

In Vitro Diagnostic Use Only
This kit contains reagents sufficient for testing of maximum of 91 specimens in a test run.

• **UUU PLATE**
  Code 5 (1x96wells)
  8 × 12/12 × 8-well per plate

MICROWELL PLATE: Blank microwell strips fixed on white strip holder. The plate is sealed in aluminium pouch with desiccant. Each well contains anti-IgM antibodies (anti-μ chain). The microwell strips can be broken to be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2-8°C. Once open, stable for one month at 2-8°C.

• **CONTROL +/-**
  Code 8 (1x0.5mL per vial)
  preserv.0.1% ProClin™ 300

NEGATIVE CONTROL: Yellowish liquid filled in a vial with green screw cap. Protein-stabilized buffer tested non reactive for anti-HBc IgM. Ready to use as supplied. Once open, stable for one month at 2-8°C.

• **Ag HRPCONJUGATE**
  Code 6 (1x12mL per vial)
  preserv.0.1% ProClin™ 300

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

CHEMICALS

-wash buffer: Colorless liquid filled in a white bottle with white screw cap.
  PH 7.4, 20 × PBS
  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**
  Code 2 (1x7mL per vial)

CHEMICALS

- **CHROMOGEN SOLUTION A**: Colorless liquid filled in a white vial with green screw cap. Urea peroxide solution.
  Ready to use as supplied. Once open, stable for one month at 2-8°C.

- **CHROMOGEN SOLUTION B**: Colorless liquid filled in a black vial with black screw cap. TMB (Tetramethyl benzidine) solution.
  Ready to use as supplied. Once open, stable for one month at 2-8°C.

- **CONTROL +/-**
  Code 7 (1x0.5mL per vial)
  preserv.0.1% ProClin™ 300

NEGATIVE CONTROL: Red-colored liquid filled in a vial with red screw cap. Anti-HBc IgM antibodies diluted in protein-stabilized buffer.
Ready to use as supplied. Once open, stable for one month at 2-8°C.
MATERIALS REQUIRED BUT NOT PROVIDED

Freshly distilled or deionized water, disposable gloves and timer, 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±0.5°C, plate reader, single wavelength 450nm or dual wavelength 450/630nm, micowell aspiration/wash system.

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 as 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. HbAb-IgM ELISA 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 7 days should be stored frozen (-20°C 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 transportation of clinical samples and ethological agents.

5. Specimen Preparation: Each specimen must be diluted 1:1000 with normal saline.

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 HBsAb-IgM ELISA, during storage, protect the reagents from contamination with microorganisms or chemicals.

PRECAUTIONS AND SAFETY

TO BE USED ONLY FROM QUALIFIED PROFESSIONALS

The ELISA assays are time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

INDICATIONS 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 results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one or contact technical support for further assistance.

INDICATIONS 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 results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one or contact technical support for further assistance.

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 three wells as Negative control (e.g. B1, C1, D1), two wells as Positive control (e.g. F1, 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.

Step2 Diluting Sample: Dilute each specimen 1:1000 with normal saline. Do not dilute the Controls, as they are ready for use as supplied.

Step3 Adding Sample: Add 100μl of Positive control, Negative control, and Specimen into their respective wells except the Blank. Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination. Mix by tapping the plate gently.

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 label 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 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 each specimen and reagents in order to avoid cross-contaminations.

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 450/630nm.

12. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.

13. If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remaining inside the plate after washing, can also be omitted.

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. WARNING: Materials from human origin may have been used in the preparation of the Negative control of the kit. These materials have been handled with high performance and found negative for antibodies to HIV 1/2, 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 to avoid any possible contamination. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.

16. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.

17. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practice) and the local or national regulations.

18. The pipette tips, vials, strips and specimen containers should be 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.

19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.

20. The Stop solution 0.5M H2SO4 is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.

21. ProClinTM 300 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.
Step 4: Incubating: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.

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

Step 6: Adding HRP-Conjugate: Add 100μL of HRP-Conjugate into each well except the Blank.

Step 7: Incubating: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.

Step 8: 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 down the plate onto blotting paper or clean towel and tap it to remove any remainders.

Step 9: 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 Positive control and anti-HBc IgM positive sample wells.

Step 10: Stopping Reaction: Using a multichannel pipette or manually, add 50μL of Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and anti-HBC IgM positive sample wells.

Step 11: 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 aspirating the liquid for 5 cycles. 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.

**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. The results are calculated by relating each specimen absorbance (A) 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 A value from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and controls.

Calculation of the Cut-off value (C.O.) = Nc × 2.1
(Nc = the mean absorbance value for three negative controls).

Important: If the mean A value of the negative controls is lower than 0.05, take it as 0.05.

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.

- The A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450nm.
- The A values of the Positive control must be ≥ 0.800 at 450/630nm or at 450nm after blanking.
- The A values of the Negative control must be < 0.100 at 450/630nm or at 450nm after blanking.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded, and the mean value should be calculated by using the remaining two values. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

**INTERPRETATIONS OF THE RESULTS**

Negative Results (A / C.O. < 1): Specimens giving A value less than the Cut-off value are negative for this assay, which indicates that no IgM-class antibodies to hepatitis B core antigen have been detected with HBCab-IgM ELISA. In case of manual washing, we suggest to carry out 5 washing cycles or soaking time per well.

Positive Results (A / C.O. ≥ 1): Specimens giving A value equal to or greater than the Cut-off value are considered initially reactive, which indicates that IgM-class antibodies to hepatitis B core antigen have probably been detected with HBCab-IgM ELISA. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for anti-HBc IgM. Positive results with anti-HBC IgM detection indicate possible recent infection with HBV.

Borderline (A / C.O. = 0.9-1.1): Specimens with A value to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results. Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system (e.g. PCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings. The result from this assay should not be used alone to establish the infection state.

INITIAL RESULTS INTERPRETATION AND FOLLOW-UP

ALL INITIALLY REACTIVE OR BORDERLINE SAMPLES

- **Sample Id:**
- **Interpretation:**
- **A/C.O.**

IND = non interpretable

- If, after retesting of the initially reactive samples, both wells are negative results (A/C.O.<0.9), these samples should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step. For more information regarding ELISA Troubleshooting, please refer to “ELISAs and Troubleshooting Guide”.

- If after retesting in duplicates, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for anti-HBc IgM.

- After retesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.
**PERFORMANCE CHARACTERISTICS**

Analytical Endpoint Sensitivity: The sensitivity of the assay has been calculated by means of the reference standards provided from the Reference Laboratory for Immunology Product under the Ministry of Health, China. The assay shows sensitivity near the Cut-off of 1 NCU (National Current Unit, MOH, China).

The clinical specificity of this assay has been determinant by a panel of samples obtained from 2500 healthy blood donors and 230 undiagnosed hospitalized patients. The repeatedly reactive samples and samples confirmed positive with the reference test were not included in the calculation of specificity.

<table>
<thead>
<tr>
<th>Specificity Number of Samples</th>
<th>-</th>
<th>+</th>
<th>Confirmed Positive</th>
<th>Sensitivity</th>
<th>False Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donors</td>
<td>2500</td>
<td>2492</td>
<td>5</td>
<td>99.87%</td>
<td>3</td>
</tr>
<tr>
<td>Patients</td>
<td>230</td>
<td>210</td>
<td>20</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>2730</td>
<td>2702</td>
<td>28</td>
<td>99.93%</td>
<td>3</td>
</tr>
</tbody>
</table>

The clinical sensitivity of this kit has been calculated by a panel of samples obtained from 548 hepatitis B patients with well-characterized clinical history based upon reference assays for detection of HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc. This panel included samples from acute, chronic and recovered hepatitis B patients. Licensed anti-HBc IgM ELISA test was used as a confirmatory assay. The evaluation results are given below.

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Number of Samples</th>
<th>-</th>
<th>+</th>
<th>Confirmed Positive</th>
<th>Sensitivity</th>
<th>False Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>315</td>
<td>4</td>
<td>311</td>
<td>99.68%</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Chronic</td>
<td>131</td>
<td>130</td>
<td>1</td>
<td>100%</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Recovery</td>
<td>102</td>
<td>101</td>
<td>1</td>
<td>100%</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Marker prevalence in follow up of patients infected with HBV:

<table>
<thead>
<tr>
<th>Days Since Infection</th>
<th>Number of Samples</th>
<th>+</th>
<th>-</th>
<th>Detected Prevalence of anti-HBc IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>20%</td>
</tr>
<tr>
<td>1-10</td>
<td>12</td>
<td>3</td>
<td>8</td>
<td>25%</td>
</tr>
<tr>
<td>11-29</td>
<td>13</td>
<td>4</td>
<td>9</td>
<td>33%</td>
</tr>
<tr>
<td>30-59</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>98%</td>
</tr>
<tr>
<td>60-89</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>90-119</td>
<td>14</td>
<td>14</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>120-149</td>
<td>11</td>
<td>11</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>150-179</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>50%</td>
</tr>
<tr>
<td>180-209</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>63</td>
<td>29</td>
<td>68.48%</td>
</tr>
</tbody>
</table>

Analytical Specificity: No cross reactivity observed with samples from patients infected with HAV, HCV, HIV, CMV, TP, and HTLV. No interferences from rheumatoid factors up to 2000U/mL were observed during clinical testing. The assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and trigleoin. Frozen specimens have been tested to check for interferences due to collection and storage.

**REFERENCES**

5. WHO/BCT/BTS/01.4. BLOOD SAFETY AND CLINICAL TECHNOLOGY. Hepatitis B Serologic Markers and Nucleic Acid Testing. NAB: Laboratory Guidelines for Screening, Diagnosis and Monitoring of Hepatic Injury Dufour, Lott, Nolte, Gretch, Koff, Seeff

**LIMITATIONS**

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immunosuppressed individuals. Therefore, negative results obtained with HBcAb-IgM ELISA are only indication that the sample does not contain detectable level of anti-HBc IgM.
3. 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. For more information regarding ELISA Troubleshooting Guide, please refer to “ELISAs and Troubleshooting Guide”, or contact technical support for further assistance.
4. The most common assay mistakes are: using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
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 kit is a qualitative assay and the results cannot be used to measure antibodies concentrations.

**SUMMARY OF THE ASSAY PROCEDURE**

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

**EXAMPLE SCHEME OF CONTROLS / SAMPLES DISPENSING**

1. Microwell plate Code 5 one
2. Negative Control Code 8 1x0.5mL
3. Positive Control Code 7 1x5mL
4. HRP-Conjugate Code 6 1x12mL
5. Wash Buffer Code 1 1x50mL
6. Chromogen Solution A Code 2 1x7mL
7. Chromogen Solution B Code 3 1x7mL
8. Stop Solution Code 4 1x7mL

**SYMBOLS**

- **<** 2°C to +8°C
- **+** Storage Conditions
- **E** Use By
- **S** Content Sufficient For <= Tests
- **K** Catalog Number
- **N** Manufacturer

**WEB PAGE**

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