HBC IgM ELISA TEST

**REF**
E0811

**IVD**

- 96-well ELISA kit for the quantitative detection of HBC IgM in human serum, plasma.
- For export only, not for re-sale in the USA.
- Store at 2°C - 8°C upon receipt.

**INTENDED USE**
The Hbc IgM ELISA Test is a solid phase enzyme linked immunosorbent assay for the qualitative detection of IgM anti-hepatitis B virus core antigen (Hbc) in human serum or plasma. It is intended for professional use only as an aid in the diagnosis of infection with HBV. Any reactive specimen with the Hbc IgM Test must be confirmed with alternative testing method(s) and clinical findings.

**INTRODUCTION**
Hepatitis virus B (HBV) is the most common cause of persistent viremia and the most important cause of chronic liver disease and hepatocellular carcinoma. Clinically apparent HBV infections may have been extant for several millennia. It is estimated that there are 300 million chronic carriers of HBV in the world. The carrier rates vary from as little as 0.3% (Western countries) to 20% (Asia, Africa).1

HBV is a hepatotropic DNA virus. The core of the virus contains a DNA polymerase, the core antigen (HbcAg) 2 and the e antigen (HbeAg) 3. The core of HBV is enclosed in a coat that contains lipid, protein and carbohydrate and expresses an antigen terms hepatitis B surface antigen (HbsAg).3

Antibody to HbcAg (anti-Hbc) appears shortly after HBsAg and before the appearance of detectable antibody to HbcAg, roughly at the time that serum ALT begins to rise. Anti-Hbc also remains elevated for life and is a useful marker of the ongoing HBV infection as HbcAg itself does not circulate freely in the serum of such infected persons.4,5

**TEST PRINCIPLE**
Hbc IgM Test is a solid phase enzyme linked immunosorbent assay based on the principle of the IgM capture technique for the detection of IgM antibody to Hbc in human serum or plasma.

The Hbc IgM Test is composed of two key components: 1) Solid microwell precoated with monoclonal anti-human IgM antibody; 2) Liquid conjugates composed of Hbc antigen conjugated with horse reddish peroxidase (HRP-Hbc Ag conjugates).

During the assay, the test specimen is first incubated with the coated microwells. IgM anti-Hbc, if present in the test specimen, binds to the antibody coated on the microwell surface.

In the second incubation with the HRP-Hbc Ag conjugates, the IgM anti-Hbc absorbed on the surface of microwell react to the HRP-Hbc Ag conjugates, forming a complexed conjugates.

Unbounded conjugates are then removed by washing. The presence of the complexed conjugates is shown by a blue color upon additional incubation with TMB substrate. The reaction is stopped with Stop Solution and absorbances are read using a spectrophotometer at 450/620/690 nm.

**MATERIALS AND REAGENTS**

**Materials and reagents provided with the kit**

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Quantity</th>
<th>Catalog</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Microwells coated with anti-human IgM antibody</td>
<td>12 wells</td>
<td>E0811W</td>
</tr>
<tr>
<td>2</td>
<td>Sample diluent (10 x concentrated)</td>
<td>10 mL</td>
<td>E0815SD</td>
</tr>
<tr>
<td>3</td>
<td>Hbc IgM negative control</td>
<td>1 mL</td>
<td>E0811N</td>
</tr>
<tr>
<td>4</td>
<td>Hbc IgM positive control</td>
<td>1 mL</td>
<td>E0811P</td>
</tr>
<tr>
<td>5</td>
<td>HRP-Hbc Ag conjugates</td>
<td>6 mL</td>
<td>E0811H</td>
</tr>
<tr>
<td>6</td>
<td>Wash Buffer (30 x concentrate)</td>
<td>20 mL</td>
<td>WE3000</td>
</tr>
<tr>
<td>7</td>
<td>TMB substrate A</td>
<td>6 mL</td>
<td>TME2000A</td>
</tr>
<tr>
<td>8</td>
<td>TMB substrate B</td>
<td>6 mL</td>
<td>TME2000B</td>
</tr>
<tr>
<td>9</td>
<td>Stop solution</td>
<td>6 mL</td>
<td>SE1000</td>
</tr>
<tr>
<td>10</td>
<td>ELISA Working Sheet</td>
<td>2 sets</td>
<td>E0011ES</td>
</tr>
<tr>
<td>11</td>
<td>Product insert</td>
<td>1 set</td>
<td>Pl-E0811</td>
</tr>
</tbody>
</table>

**Materials and reagents required but not provided in the kit**

1. Pipette capable of delivering 1 ul, 50 mL, 100 mL, and 1000 mL volumes with a precision better than 1.5%.
2. Test tubes for specimen dilution.
3. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-3.0D or greater at 450nm wavelength is acceptable.
4. Absorbtion paper for blotting the microplate wells.
5. Parafilm or other adhesive film sealant for sealing plate.
6. Timer.
7. Distilled or de-ionized water.

**STORAGE AND STABILITY**

All reagents except the concentrated wash buffer are ready to use as supplied. Return all reagents requiring refrigeration immediately after use. Reseal the microcwell after removing the desired number of wells. Ensure that the reagents are brought to room temperature before opening. All the reagents are stable through the expiration date printed on the label if not opened. Do not freeze the kit or expose the kit over 8°C.

**WARNING AND PRECAUTIONS**

For in Vitro Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not use expired devices.
3. Bring all reagents to room temperature (18°C-28°C) before use.
4. Do not use the components in any other type of test kit as a substitute for the components in this test.
5. Do not use hemolized blood specimen for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mucous. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Dispose of all specimens and materials used to perform the test as biohazardous waste.

In the beginning of each incubation and after adding Stopping Solution, gently rocking the microwells to ensure thorough mixing. Avoid the formation of air bubbles as which results in inaccurate absorbance values. Avoid splash liquid while rocking or shaking the wells.

Don’t allow the microplate to dry between the end of the washing operation and the reagent distribution.

The enzyme reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or substrate solution.

13. The substrate solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The Substrate B must be stored in the dark.

Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.

15. The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.

Avoid strong light during color development.

**SPECIMEN COLLECTION AND PREPARATION**

- Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
- This kit is designed for use with serum or plasma specimen without additives only.
- If a specimen is not tested immediately, refrigerated at 2°C-8°C, if storage period greater than three days are anticipated, the specimen should be frozen (−20°C). Avoid repeated freezing-thawing of specimens. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assay.
- Do not use serum specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

**PREPARATION OF THE REAGENTS**

1. Bring all reagents, controls to room temperature (18°C-28°C).
2. Dilute concentrated Wash Buffer 30 fold with water as following:

<table>
<thead>
<tr>
<th>Plate</th>
<th>DI water</th>
<th>30 X wash buffer</th>
<th>Final volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full plate</td>
<td>580 mL</td>
<td>20 mL</td>
<td>600 mL</td>
</tr>
<tr>
<td>Half plate</td>
<td>290 mL</td>
<td>10 mL</td>
<td>300 mL</td>
</tr>
<tr>
<td>A quarter plate</td>
<td>145 mL</td>
<td>5 mL</td>
<td>150 mL</td>
</tr>
</tbody>
</table>

Warm up the concentrated Washing Buffer at 37°C to dissolve the precipitant if it appears.

3. Concentrated Sample Diluent contains 9% NaCl. Dilute it with water prior to use as following:

Add 10 mL of the concentrated Sample Diluent into 90 mL of water and mix well. The working sample diluent contains 0.9% NaCl. Alternatively, user can use Saline Buffer as the sample diluent.

4. Dilute test specimen with the working sample diluent at 1:1000 dilution, ie: 1 mL of test serum or plasma into 1000 mL of the working sample diluent.

5. Determine the number of microwells needed and mark on the ELISA Working Sheet with the appropriate information. Positive and Negative Controls require to be run in duplicate to ensure accuracy.

6. Mix each reagent before adding to the test wells.
**ASSAY PROCEDURE**

1. Remove the desired number of strips and secure them in the microwell frame. Re-seal un-used strips.

2. Add specimens according to the designation on the ELISA Working Sheet

   2.1 **Blank well**: Leave the blank well alone. Don’t add any reagents.

   2.2 **Control wells**: Add 50 µL of HBc IgM Positive, Negative Control into the designated control wells, respectively.

   2.3 **Test wells**: Add 50 µL of diluted test specimens into each test well, respectively.

   To ensure better precision, use pipette to handle solution.

3. Incubate the wells at 37°C for 30 minutes.

4. Carefully remove the incubation mixture by emptying the solution into a waste container. Fill each well with diluted wash buffer and shake gently for 20-30 second. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 4 more times.

5. Add 50 µL of HRP-HBc Ag conjugate into each well except the blank well, cover the plate, and incubate at 37°C for 30 minutes.

6. Wash the plate 5 times as step 5 described.

7. Add 50 µL (or 1 drop) of TMB substrate A and 50 µL (or 1 drop) of TMB substrate B into each well including the blank well.

8. Incubate at 37°C in dark for 10 minutes.

9. Stop the reaction by adding 50 µL (1 drop) of stop buffer to each well. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

11. Set the microplate reader wavelength at 450 nm and measure the absorbance (OD) of each well against the blank well. Subtract the OD of each test well from the OD of the blank well.

**INTERPRETATION OF RESULTS**

**A. Set up the cut-off value**

The cutoff value = N x 2.1  
N: Mean OD of the negative control. Use 0.05 for calculation of the cut-off value if the mean OD is less than 0.05.

**B. Calculation of specimen OD ratio**

Calculate an OD ratio for each specimen by dividing its OD value by the cut-off value as follows:

Specimen OD ratio = -----

Cut-off Value

**C. Assay validation**

The mean OD value of the HBc IgM positive controls should be ≥ 0.80. The mean OD value of the HBc IgM negative controls should be ≤ 0.10. Check the procedure and repeat assay if above conditions are not met.

**D. Interpretation of the results**

**Specimen OD ratio**

<table>
<thead>
<tr>
<th>OD Ratio</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>&lt; 1.00</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>≥ 1.00</td>
<td></td>
</tr>
</tbody>
</table>

1. The negative result indicates that there is no detectable IgM anti-HBc in the specimen.

2. Results just below the cut-off value (Lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to retest in duplicate the corresponding specimens when it is applicable).

3. Specimens with cut-off ≥ 1.00 are initially considered to be positive by the HBc IgM Test. They should be retested in duplicate before final interpretation.

   If after re-testing of a specimen, the absorbance value of the 2 duplicates are less than the cut-off value, the initial result is non-repeatable and the specimen is considered to be negative with the HBc IgM Test.

   Non-repeatable reactions are often caused by:

   - Inadequate microwell washing.
   - Contamination of negative specimens by serum or plasma with a high antibody titer.
   - Contamination of the substrate solution by oxidizing agents (bleach, metal ions, etc.)
   - Contamination of the stopping solution

   If after re-testing the absorbance of one of the duplicates is equal or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the HBc IgM Test, subject to the limitation of the procedure, described below.

**PERFORMANCE CHARACTERISTICS**

**Clinical Performance**

A total of 400 specimens from susceptible subjects were tested by a Chinese State Drug Administration (SDA) licensed reference EIA. Comparison for all subjects is shown in the following table:

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref. HBc IgM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>46</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>354</td>
<td>354</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>354</td>
<td>400</td>
</tr>
</tbody>
</table>

Relative Sensitivity: 100%, Relative Specificity: 100%, Overall Agreement: 100%

**LIMITATION OF THE TEST**

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of HBc IgM in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.

2. The HBc IgM Test is limited to the qualitative detection of IgM antibodies to HBc in human serum or plasma. The intensity of color does not have linear correlation with the antibody titer in the specimen.

3. A negative result for an individual subject indicates absence of detectable IgM anti-HBc. However, a negative test result does not preclude the possibility of exposure to or infection with HBV.

**REFERENCES**


**Index of Symbols**

- [ ] consult see instructions for use
- [ ] For in vitro diagnostic use only
- [ ] Catalog #
- [ ] Lot Number
- [ ] Use by
- [ ] Tests per kit
- [ ] Store between 2-8°C
- [ ] Do not reuse
- [ ] Manufacturer
- [ ] Date of manufacture

**Manufacturer**

CTK Biotech, Inc.  
10110 Mesa Rim Road  
San Diego, CA 92121, USA  
Tel: 858-457-6698  
Fax: 858-535-1739  
E-mail: info@ctkbiotech.com  
PL-E0811. Rev. C.  
Effective date: 2011-05-10