Human HBV Core antigen / HBcAg antibody ELISA Kit

Human HBV Core antigen / HBcAg antibody ELISA Kit is an Enzyme Immunoassay kit for the determination of Human HBcAg antibody in serum and plasma (EDTA, heparin, citrate).

Catalog number: ARG82306

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

HBcAg (core antigen) is a hepatitis B viral protein. It is an indicator of active viral replication; this means the person infected with Hepatitis B can likely transmit the virus on to another person.

HBcAg is an antigen that can be found on the surface of the nucleocapsid core (the inner most layer of the hepatitis B virus). While both HBcAg and HBeAg are made from the same open reading frame, HBcAg is not secreted. HBcAg is considered "particulate" and it does not circulate in the blood but recent study show it can be detected in serum by Radioimmunoassay. However, it is readily detected in hepatocytes after biopsy. The presence of both HBcAg and HBeAg proteins together act as a marker of viral replication, and antibodies to these antigens are a marker of declining replication. [Provide by Wikipedia: HBcAg]

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. The solid phase of the microplate is made of polystyrene wells coated with HBcAg and the liquid phase of human peroxidase conjugate Anti-HBc. When a specimen containing Anti-HBc is added to the HBcAg coated plate together with the human peroxidase conjugated Anti-HBc and incubated, a competition will take place for the binding to the HBcAg on the wells. After washing the plate to remove unbound material, the TMB Substrate is added to the wells and incubated. Due to the competitive principle a color develops inversely proportional to the amount of Anti-HBc bound to HBcAg deriving from the specimen. Then the Stop Solution is added to the wells to stop the reaction.

The optical density of developed color is read with a plate reader at O.D. 450 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Do not freeze. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen Coated Microplate	96 wells	4°C
Anti-HBc·Peroxidase Solution	8 mL (ready to use)	4°C
Anti-HBc Positive Control	1.5 mL (ready to use)	4°C
HB Negative Control	2.0 mL (ready to use)	4°C
20X Wash Buffer	58 mL	4°C
TMB Substrate A	12 mL	4°C (protect from light)
TMB Substrate B	12 mL	4°C (protect from light)
Stop Solution	12 mL (ready to use)	4°C

Note:

- Adhesive Slips / Absorbent Pads / Black Cover provide as needed.
- All component except Antigen Coated Microplate are stable for up 1 month once open. (Antigen Coated Microplate is stable for up 2 month once open)

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620-690 nm as optional reference wave length)
- Centrifuge and centrifuge tube
- Incubator (37°C)
- Deionized or distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Although all human sourced material are tested non-reactive for Anti-HCV and Anti-HIV, and inactivated at 56°C for one hour, the reagent shall be handled as potential infectious material.
- Return any unused microplate strips to the plate pouch with desiccant.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-30°C).
- The reagent preparation method might be different from lot to lot, so please check the lot and follow the instructions given in this manual.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.

- If crystals are observed in the 20X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C.

Note: (see next page)

- Whole blood specimen should be separated as soon as possible in order to avoid hemolysis.
- Specimens must be stored at 4°C and avoided heat inactivation to minimize deterioration.
- For long term storage, specimens should be frozen below -20°C. Storage in self-defrosting freezers is not recommended.
- Avoid multiple freeze-thaw procedures.
- The specimen must not contain any AZIDE compounds which can inhibit the peroxidase activity.
- Incompletely coagulated serum samples and microbial-contaminated specimens should not be used.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 20X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 25 mL of 20X Wash Buffer into 475 mL of distilled water to a final volume of 500 mL). 1X Wash Buffer is stable at 4°C for up to 1 week.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-30°C) before use. Standards and samples should be assayed in duplicates.

1. Reserve 2 wells for blanks. Add 50 µL of each control or sample to Antigen Coated Microplate. (3 Negative Controls and 2 Positive Controls)

   Note: Do not use cut-off value established for other plates of Human HBV

Core antigen / HBcAg antibody ELISA Kit.

2. Add **50 µL** of **Anti-HBc·Peroxidase Solution** to each well except the 2 blanks.
3. Gently tap the plate. Cover the plate and incubate for **1 hour** at **37°C.**
4. Remove plate cover. Aspirate each well and wash, repeating the process 7 times for a total 8 washes. Wash by filling each well with **1X Wash Buffer (350 µL)** using a squirt bottle, manifold dispenser. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **50 µL** of **TMB Substrate A** first, then add **50 µL** of **TMB Substrate B** to each well including the 2 blanks. Mix well gently.
6. Cover the plate and incubate for **30 minutes** at **room temperature** in the dark.
7. Immediately Add **100 µL** of **Stop Solution** to each well including the 2 blanks. The color of the solution should change from blue to yellow.
8. Read the absorbance at **O.D. 450 nm**. (with **620-690 nm** reference wavelength)

   Note: absorbance value of blank wells must be less than **0.100.**

CALCULATION OF RESULTS

1. Calculation of the NCx (Mean Absorbance of Negative Control).

   Example:

Sample No.	Absorbance
1	0.939
2	0.944
3	0.925

   NCx = (0.939 + 0.944 + 0.925) / 3 = 0.936 (**NCx must be ≥ 0.4, otherwise, the test run is invalid**).

2. Calculation of the PCx (Mean Absorbance of Positive Control).

   Example:

Sample No.	Absorbance
1	0.068
2	0.052

   PCx = (0.068 + 0.052) / 2 = 0.060 (**PCx must be ≤ 0.1, otherwise, the test run is invalid**).

3. Calculation of the N – P value

   Example:

   N – P = NCx – PCx = 0.936 – 0.060 = 0.876 (**N-P value must be ≥ 0.3, otherwise, the test run is invalid**).

4. Calculation of the Cutoff Value

   Cutoff Value = 0.4 NCx + 0.6 PCx

   Example: Cutoff Value = (0.4 x 0.936) + (0.6 x 0.060) = 0.410

5. Calculation of the Retest Range

   Retest Range = Cutoff Value ± 10%

Example: Cutoff Value = 0.410,

Retest Range = (0.410 – 0.041) to (0.410 + 0.041) = 0.369 to 0.451

INTERPRETATION OF RESULT

If the signal / Cut-off ration is within Retest Range, the test must be repeated in duplicate and interpreted as above. IF both results are non-reactive the final result is non-reactive, if both result are reactive the final result is reactive. Any other combination is an indeterminate result. Testing of follow up sample and other hepatitis B serological markers should be taken into account in case of an indeterminate result.

QUALITY ASSURANCE

Sensitivity

1.869 PEI U/mL