

HBc IgM ELISA Kit

Enzyme-linked immunosorbent assay for the detection IgM antibody against HBcAg in serum or plasma

INTENDED USE

Anti-HBcAg antibody (HBcAb) IgM EIA is a qualitative enzyme immunoassay for the detection of IgM antibody to core antigen of hepatitis B virus (HBc) in human serum or plasma. It is a IgM capture assay that features high specificity with a simple and fast procedure. This assay is designed to distinguish the acute and chronic status of hepatitis B infection.

SUMMARY AND PRINCIPLE OF THE TEST

Hepatitis B core Antigen (or HBcAg) is the major component of the core particles of Hepatitis B virus (or HBV). Particles have a size of 27 nm and contain a circular double-stranded DNA molecule, a specific DNA-polymerase and HBcAg. HBcAg is composed of a single polypeptide of about 17 kD that is released upon disaggregation of the core particles; the antigen contains at least one immunological determinant. Upon primary infection, anti HBcAg IgM antibodies are one of the first markers of HBV hepatitis appearing in the serum of the patient, together or slightly later than HBsAg, the viral surface antigen. Anti HBcAg IgM titers, very high during the acute phase, decrease along the illness, as IgG antibodies appear, down to undetectable levels in convalescent patients. In chronic hepatitis, however, spikes of anti HBcAg IgM synthesis are present, confirming reactivation of HBV in hepatocytes and giving origin to permanent IgM low titers. The determination of anti HBcAg IgM antibodies has become very important for the fast classification of the virus, of the phase of the illness and for the monitoring of patients under treatment with interferon.

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody. After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a purified preparation of recombinant HBcAg, labelled with a monoclonal antibody conjugated with peroxidase (HRP). After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added. In the presence of peroxidase the colourless substrate is hydrolysed to a coloured end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to HBcAg present in the sample.

REAGENTS

Materials provided with the kits:

1. Microtiter Well: coated with goat anti-human IgM.
2. Negative Control: 0.25ml normal serum.
3. Positive Control: 0.25ml anti-HBcAg IgM positive serum.
4. Enzyme Conjugate: 6 ml, monoclonal anti-HBcAg-HRP with HBcAg antigen immunocomplex.
5. Wash Buffer Concentrate (20x): 40 ml, The buffer should be diluted 20 times with distilled water before use.
6. Substrate Solution A: 6 ml Urea Peroxide
7. Substrate Solution B: 6 ml TMB solution
8. Stop Solution: 6 ml 2N Sulfuric Acid

Materials required but not provided:

1. Precision pipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Plasma samples containing EDTA, heparin or oxalate may interfere with test procedures and should be avoided. Specimen with extensive particulate should be clarified by centrifugation prior to use. Covered specimens may be stored for up to 48 hours at 2°-8°C prior to assaying. Specimens held for a longer time can be frozen at -20°C for mix prior to testing. Avoid repeated freeze thaw. At least, two wells of negative and positive controls each should be run in every assay.

PRECAUTIONS

1. Caution: Some components of this kit contain human serum. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
2. Wear disposable gloves while handling kit reagents and specimens and thoroughly wash hands afterwards.
3. Dispose off all specimens and materials used to perform the test as if they contained infectious agents.
4. Do not mix reagents from kits with different lot numbers.
5. Cross contamination between reagents will invalidate the test results.
6. All reagents and components except the conjugate must be equilibrated at room temperature prior to use.

STORAGE OF TEST KITS AND INSTRUMENTATION

Unopened test kits should be stored at 2°-8°C upon receipt. Micro titer plate, once opened, should be kept in a sealed bag with desiccants to minimize exposure to damp air. To remove the required number of strips from the micro titer plates, bring the sealed pouches to room temperature first and then open the pouches. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. Opened test kits will remain stable until the expiration date shown in 4°C, provided it is stored as described above. A micro titer plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

WORKING REAGENT PREPARATION, STORAGE AND STABILITY

No reagent preparation is required except for wash buffer, which is supplied as a 20 X concentrate.

WORKING WASH BUFFER

Dilute the 20X wash buffer concentrate with deionized or distilled water 1:20. For example, 5 ml of wash buffer concentrate should be diluted to a total volume of 100 mL with deionized or distilled water.

STABILITY OF OPENED KIT COMPONENTS AND DILUTED REAGENTS

The diluted wash buffer is stable for at least one week when stored at room temperature. Substrate is stable for the expiration date of the kit. The micro titer plates should be opened after they have been kept at room temperature for 20-30 minutes. After removing the required number of strips, the plates should be resealed in the foil pouch bags along with the desiccant and stored at 2°-8°C. Exposure of the plates to humidity drastically reduces the shelf life.

ASSAY PROCEDURE:

1. Allow all reagents to reach room temperature before use.
2. Dispense 50µl of 1:1000 1X washing buffer-diluted specimen into each well.
3. Dispense 50µl Positive Control as well as Negative Control in duplicate into respective wells (do not dilute controls). Set one black well as background control.
4. Incubate at 37°C humidified box for 30 min.
5. Wash each well 4 times by filling each well with diluted 1X wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
6. Add 50µl of Enzyme Conjugate to each well. Mix them gently by swirling the microtiter plate on flat bench for 1 min. Do not add Antigen and Enzyme Conjugate to the blank well. 100ul of washing buffer added to the blank well.
7. Incubate at 37°C for 30 min.
8. Wash 4 times as step 4.
9. Add one drop (50 ul) of Substrate Solution A to each well, then add one drop (50 ul) of Substrate Solution B to each well. Mix gently and incubate at 37°C for 10 min.
10. Visual inspection of the color reaction in each well or add one drop (50 ul) of Stop Solution to each well to stop the color reaction. Blank EIA plate reader with the blank control well and then read O.D. values of all samples at 450 nm.

INTERPRETATION OF RESULTS

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):

Positive: P/N value is equal to or greater than 2.1

Negative: P/N value is less than 2.1

$$P/N \text{ value} = \frac{\text{OD value of specimen}}{\text{Average OD value of Negative Control}}$$

If the OD value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual OD value measured.

LIMITATIONS OF THE ASSAY

1. HBcAb IgM EIA is limited to the detection of IgM antibody against HBcAg in serum or plasma.
2. As in other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. So do aspirate the well or get rid of the entire content of wells completely before adding the washing solution.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be made based only on the results of a single test. A complete evaluation by physician is needed for a final diagnosis.
4. Do not use reagents from different tests that will cause incorrect results.
5. Following the procedure instruction closely, especially the incubation time and temperature.











RELATED READING MATERIALS

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2. Blumberg B.S. et al., Australia antigen and hepatitis. *New Eng. J. Med* 283:349-354 (1970)
3. Hoofnagle J.H. et al., Antibody to Hepatitis B virus I core in man. *Lancet* 11:869-873, (1973)
4. Szmuness, W. et al. Antibody against the hepatitis type B core antigen. *Am. J. Epidemiol.* 104: 256-262 (1976)

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|  | <i>In vitro</i> diagnostic device |  | Lot code |
|  | Consult instructions for use |  | Catalogue number |
|  | Keep dry |  | Contains sufficient for <n> tests |
|  | Temperature limitation |  | Manufacturer |
|  | Use by |  | Do not use if package damaged |