DRG® HBc IgM (EIA-4085)

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INTENDED USE
An Enzyme ImmunoAssay (ELISA) for determination of IgM class antibodies to Hepatitis B Virus core Antigen in plasma and sera with the "capture" system. For Research Use Only.

PRINCIPLE OF THE ELISA TEST
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 colorless substrate is hydrolyzed to a colored end product, whose optical density may be detected and is proportional to the amount of IgM antibodies to HBcAg present in the sample.

COMPONENTS
Antibody coated microwells:
8x12 microwell strips coated with purified anti hIgM antibody and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

Standard Curve:
1x2.0 ml/vial. Ready to use standard curve ranging: 0-5-10-20-50-100 PEI U/ml. Contains human serum proteins, 0.09% sodium azide and 0.3 mg/ml gentamicine sulphate as preservatives.

Wash buffer concentrate:
1x60 ml/bottle. 20x concentrated solution to be diluted up to 1200ml-distilled water before use. Contains phosphate buffer and Tween 20. Once diluted, the wash solution is stable for 1 week at 2-8° C .

Immunocomplex: **(CONJ 10X)**
1x1.6 ml/vial. 10x concentrated solution. Contains an immunocomplex formed by a specific monoclonal antibody, labeled with HRP, and a purified recombinant HBcAg. The reagent is dissolved into a buffer solution containing 0.3 mg/ml gentamicine sulfate and 0.1% Kathon GC as preservative.

Immunocomplex Diluent:**(CONJ DIL)**
1x16 ml/vial. Buffered proteic solution for the dilution of the concentrated Immunocomplex. Contains 0.3 mg/ml gentamicine sulfate and 0.1% Kathon GC as preservative.

Sample Diluent : **(DILSPE)**
2x60.0 ml/vial. Proteic buffered solution for the dilution of samples; contains a detergent, a blue dye and sodium azide as preservative.

Chromogen: (SOLN TMB)
1x8ml/vial. Contains a buffered solution of stabilized TMB or tetramethylbenzidine. **Note:** To be stored protected from light.

Substrate: (SOLN H2O2)
1x8ml/vial. Contains a buffered solution of stabilized H_2O_2 or hydrogen peroxide.

Stop solution:
1x15ml/vial. Contains 0.3 M H_2SO_4 solution. Warning: Avoid contact with eyes or skin. Irritant.

Plate sealing foil
Instruction manual

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micropipettes and disposable tips
2. EIA grade water.
3. Timer with 60-minute range.
4. Absorbent paper.
5. Microplate thermostatic incubator set at +37°C
6. Microwell reader with 450nm and 620-630nm filters.
7. Microplate washer

PRECAUTIONS

1. Upon receipt, store the kit at 2-8°C.
2. Do not interchange components between different kits.
3. Check that the reagent solutions are clear.
4. Avoid cross-contamination between serum specimens.
5. Avoid cross-contamination between reagents.
6. Do not use the kit after the expiration date.
7. Treat all specimens and kit reagents as potentially infectious. Although the human sera utilized in preparing the kit reagents have been tested and found to be negative for HBsAg, HCV Ab and HIV Ab, there is no test method available that can offer complete assurance that hepatitis B virus, HIV or other infectious agents are absent.
   Therefore all human serum specimens and kit reagents should be handled at the Biosafety Level 2 as recommended by the Centers for Disease Control/U.S. Institutes of Health publication "Biosafety in Microbiological and Biomedical Laboratories", 1984.
8. Never pipette by mouth, and use gloves when handling human blood or serum specimens and other potentially infectious materials.
9. The use of disposable glass or plastic-ware is recommended in order to avoid contamination.
10. The washing waste and all used reagents should be discarded into a disinfectant solution such as sodium hypochlorite (i.e. 50ml household bleach in 950ml water) before disposal.
11. Other materials should be treated as biohazard waste and also disposed according to recommended procedures.

SPECIMEN PREPARATION

Aseptically draw blood by venipuncture and collect the serum using standard techniques. Avoid using hemolyzed or hyperlipemic sera, which may give erroneous results. Serum specimens can be stored at 2°C to 8°C for up to five days after collection. For longer periods the specimen should be stored frozen at –20°C. In this case, do not freeze and thaw samples more than once as IgM antibodies may get damaged and denaturated. Avoid any addition of preservatives, particularly sodium azide which may inhibit the enzyme reaction.

PREPARATION OF REAGENTS

a. **Washing Solution:** the concentrated solution has to be diluted 20x in ELISA grade water and mixed before use.

b. **Immunocomplex:** dilute the 10x concentrated Immunocomplex 1:10 with the proper diluent and mix carefully before use.
c. **Chromogen/Substrate:** about 5 minutes before use, prepare this reagent in a disposable plastic tube, according to need, by mixing 1 volume of Chromogen with 1 volume of Substrate.

ASSAY PROCEDURE

1. Place the required number of strips in the microplate holder. Leave the 1st well empty for the operation of blanking. Store the other strips into the bag in presence of the desiccant at +4°C, sealed.
2. Dilute samples 1:101 dispensing 1 ml Sample Diluent into a disposable tube and 10 ul sample; mix on vortex before use. Do not dilute standards as they are ready-to-use.
3. Pipette 100µl standards in duplicate and then 100ul of samples. Incubate the microplate at +37°C for 60 min.
4. Wash the microplate with an automatic washer by delivering and aspirating 300ul/well of diluted washing solution for five times with soaking. **Important note:** The washing procedure is essential for the assay to provide reliable and precise results. The instrument has to be correctly serviced and maintained at the best of its performances. The right washing procedure has to be experimentally found and checked for the specific kit in use by matching the values of quality control reported below.
5. In all the wells except A1, pipette 100 µl diluted Immunocomplex. Incubate the microplate at +37°C for 60 minutes.
6. Wash the microplate as described.
7. Pipette 100µl TMB/H_2O_2 mixture, prepared as described, in each well, the blank well included. Then incubate the microplate at room temperature for 20 minutes.
8. Pipette 100µl Stop Solution into each well using the same pipetting sequence as in step 7. Then measure the color intensity of the solution in each well using a microwell reader at 450nm filter (reading) and possibly at 620-630nm (blanking), blanking the instrument on the 1st well.

ASSAY SCHEME

Standards Samples diluted 1:101	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
TMB/H_2O_2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Stop solution	100 ul
Reading OD	450nm

RESULTS

If the test turns out to be valid, elaborate the standard curve with a qualified curve fitting system and then calculate the concentration of samples on the curve.

Example of standard curve

0 U/ml 0.050 OD450nm
5 U/ml 0.200 OD450nm

10	U/ml	0.350	OD450nm
20	U/ml	0.600	OD450nm
50	U/ml	1.000	OD450nm
100	U/ml	1.700	OD450nm

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