Version 21-04/24

User's Manual

Hepatitis B Surface Antigen ELISA Kit

REF **DEIA001**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

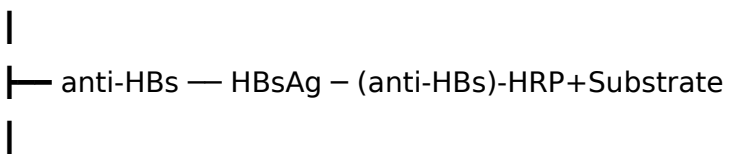
1. For screening of blood donors.
2. As an aid in the diagnosis of liver disease.

General Description

CD HBsAg is an in vitro diagnostic kit for the detection of hepatitis B surface antigen (HBsAg) in human serum or plasma.

Principles of Testing

The test is an enzyme-immunoassay based on a sandwich principle.



solid-phase positive enzyme-labeled

antibody sample antibody

Polystyrene microtiter strip wells have been coated with monoclonal anti-HBs(antibody to HBsAg), which constitutes the solid-phase antibody. The test sample is incubated in such a well; HBsAg, if present in the sample, will bind to the solid-phase antibody. Subsequently anti-HBs, which has been labeled with the enzyme horseradish peroxidase (HRP), is added. With a positive reaction this labeled antibody becomes bound to any solid-phase antibody HBsAg complex previously formed. Incubation with enzyme substrate produces a blue color in the microwell, which turns yellow when the reaction is stopped with sulphuric acid. If the sample contains no HBsAg, the labeled antibody cannot be bound specifically and only a low background color develops.

Reagents And Materials Provided

1. Coated Microplate: 1 plate (96 tests), twelve 8-well strips per plate. Each microplate well is coated with plate monoclonal anti-HBs (mouse), and sealed in an aluminum bag containing a silica gel bag as desiccant.
2. Conjugate: 1 vial of 6.2ml (HRP-labeled anti-HBs).
3. Positive Control: 1 vial of 1.0ml.
4. Negative Control: 1 vial of 1.0ml.
5. Washing solution: 1 bottle of 40 ml concentrated, which must be diluted 25-fold before use.
6. Chromogen A: 1 vial of 8.0ml (contains hydro-peroxide).
7. Chromogen B: 1 vial of 8.0ml (contains TMB).
8. Stopping solution: 1 vial of 7.0ml (0.5M sulphuric acid).
9. Plate sealers: 2 pieces.

10. Instruction manual: 1 copy.

Materials Required But Not Supplied

1. Distilled water.
2. Manual or automatic pipettors capable of delivering 20µL, 100µL, 1000µL; the pipettors should not contain metal parts that can come into contact with the liquid.
3. Disposable pipette tips.
4. Timer.
5. Microplate mixer.
6. Incubator 37°C.
7. Microplate washer (alternatively, washing can be performed manually, e.g. by using a repeating syringe delivering 0.3 ml volumes).
8. Microplate reader equipped with a 450nm and 630nm filter.
9. Gloves.

Storage

1. If kept at 2 to 8°C, all the test reagents are stable until the expiry date printed on the kit.
2. When the aluminum bag has been opened, the unused strips can be safely stored at 2-8°C in the sealable plastic pouch along with the silica gel placed inside for about two weeks.

Specimen Collection And Preparation

1. Serum or plasma should be free of microbial contamination when tested.
2. Sample should not be added sodium azide (NaN3) as a preservative.
3. Additives (other than gentamic in sulfate or proclin) and repeated freezing and thawing may give erroneous results.
4. Precipitates, clots and blood cells may cause an increased number of false positive results. Therefore insoluble material should be removed from all samples by centrifugation before testing.

Reagent Preparation

1. Dilute the concentrated wash solution 1:25 with distilled water. The diluted wash solution must be at 20 to 25°C when used.
2. Allow the test samples, controls, conjugate, diluted wash solution, aluminum bag containing the microplate and Chromogen A and B to come to the room temperature before use.

Assay Procedure

1. Open the aluminum bag and take out the microplate with the required number of strips. The left strips are placed in the plastic pouch along with the silica gel bag, and sealed (see Storage and stability). During the

test, the strips must stay in the microplate.

2. Pipette 75µL of each specimen into the wells (leave 5 wells for controls and blank) pipette 50µL of Positive Control into each of the two wells, and 75µL of Negative Control into each of the two wells, and remain one blank, following the addition of the samples.
3. Cover the strips with a plate sealer. Incubate at 37°C for 60 minutes.
4. Pipette 50µL of conjugate into each well (excluding the blank well).
5. Cover the strips with a plate sealer. Incubate at 37°C for 30 minutes.
6. During incubation, dilute the concentrated washing solution 1:25.
7. Wash each well with the solution above five times and then blots dry by pressing plate onto absorbent tissue.
8. Pipette 50µL of Chromogen A into each well (including the blank well).
9. Pipette 50µL of Chromogen B into each well (including the blank well).
10. Cover the plate with a fresh plate sealer. Incubate at 37°C for 15 minutes in an incubator.
11. Stop the reaction by adding 50µL of stopping solution to each well (including the blank well) and mixing completely.
12. Microplate reading: Put the plate in the microplate reader and read the absorbance of the solution in the wells at 450nm and 630nm.

Calculation

The judgment of results is based on the photometric reading data.

Abbreviations

N = the mean absorbance of the negative controls

P = the mean absorbance of the positive controls

S = the absorbance of the test sample

Set the blank well you choose as blank, read the absorbance of the other wells.

Calculation of cut-off value: The cut-off value is 0.100+N.

Test result:

A specimen is negative if S is less than the cut-off value.

A specimen is positive if S is greater than or equal to the cut-off value.

Checking of test-run validity: A test-run is only valid if N<0.1 and P>1.0.

Interpretation Of Results

A negative result means that the sample tested either contains no HBsAg or contains HBsAg below the detection limit of CD HBsAg. A positive result means that the sample tested either contains HBsAg or a nonspecifically reacting factor. As with other immunoassays, occasional false positive reactions may occur, which are in most instances non-repeatable. It is therefore recommended to re-test all samples giving an initially positive result. Only a repeatable positive result should be considered reactive for HBsAg.

Sensitivity

Human Anti-HBsAg ELISA Kit

Precautions

1. In one screening test-run do not mix strips, conjugate and controls from kits with different lot numbers.
2. Do not perform the test in the presence of reactive vapors (e.g. from acids, alkalis or aldehydes) or dust, since the enzymatic activity of the conjugate may be affected.
3. All vials and bottles used for preparing Chromogen must be cleaned thoroughly and finally rinsed with distilled water.
4. To avoid contamination, do not touch the edges of the wells with the pipette tips when adding sample, conjugate or substrate.
5. To avoid contamination, do not touch the top of the strips with your fingers.
6. All pipetting steps should be performed with the utmost care and accuracy.
7. Solutions containing TMB and/or peroxide should not come into contact with metals or metal-ions, since this may give rise to unwanted color formation.
8. If the wells cannot be filled with Chromogen immediately after washing, the microplate may be placed face down on a wet absorbent tissue for not longer than 15 minutes.

Caution

The positive control contains HBsAg. The negative control is derived from human blood prepared only from donations which have been tested individually for HBsAg as well as for Anti-HIV(I+II) antibodies and Anti-HCV antibodies by reliable methods and found to be negative. However, as no test method can offer complete assurance that infectious agents are absent, all specimens of human sourced should be considered potentially infectious and handled with gloves.

Chromogen B contains Dimethyl Sulphoxide, an irritant to skin and mucous membranes. (Avoid inhaling the vapors)

Dispose of all specimens and materials used to perform the test as if they contained infectious agents. Microplate and equipment should be disinfected after use. The preferred method of disposal is autoclaving for half hour at 121°C or above.