Human Anti-Hepatitis C Virus Antibody, Anti-HCV ELISA Kit

Cat. No.:DEIA015
Pkg.Size:96T

Intended use

The Human Anti-Hepatitis C Virus Antibody, Anti-HCV ELISA Kit is designed for the detection of antibodies to Hepatitis C virus in human serum or plasma.

General Description

HCV is the etiological agent responsible for the disease known as Non-A, Non-B Hepatitis, or Hepatitis C. The clinical symptoms of Hepatitis C are jaundice, acute liver hepatitis, and elevated liver enzymes. Roughly half of the individuals who contact the disease develop chronic hepatitis, and 20% develop liver cirrhosis and an increase risk for developing liver cancer. The virus is largely transmitted via contaminated blood products by blood transfusion, hemodialysis and intravenous drug use.
This immunoassay employs both synthetic and recombinant HCV antigens for the detection of antibodies to HCV in human serum or plasma. These antigens correspond to the conserved epitopes of the virus.
Specimens with absorbances greater than or equal to the cutoff value are defined as initially reactive. Initially reactive specimens MUST BE re-tested to exclude the possibility of a cross-reaction. Specimens, which are reactive in a duplicate test, are defined as repeatable reactive. In diagnosing HCV such specimens must be confirmed as HCV positive by using a western blot test. Laboratories should follow the guidelines of their own national public health ministry.

Principle Of The Test

Polystyrene microtiter strip wells have been coated with HCV specific antigens derived from "core" and "ns" regions encoding for conservative immunodominant antigenic determinants. The diluted sample is incubated in such a well; antibodies to HCV are captured, if present, by the antigens during the time of incubating. After washing out the unbound components of the samples, the anti-Human IgG antibodies, labeled with peroxidase (HRP) are added to the wells. The enzyme conjugates will bind to the complex and excess unbound enzyme conjugates are removed by washing again. 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 antibodies to HCV, the labeled antibody cannot be bound specifically and only a low background color develops. antibodies to HCV.

Reagents And Materials Provided

1. Coated Microplate: 1 plated (96 tests),twelve 8-well strips per plate. Each microplate well is coated with HCV specific antigen, and sealed in an aluminum bag containing a silica gel bag as desiccant.
2. Conjugate: 2 bottles 7.5ml
3. Positive control: 1 vial of 1.0ml
4. Negative control: 1 vial of 1.0ml
5. Wash Solution: 1 bottle of 80ml concentrated; which must be diluted 1:25 before use.
6. Chromogen A: 1 vial of 8ml (contains hydro-peroxide).
7. Chromogen B: 1 vial of 8ml (containing TMB).
8. Stopping Solution: 1 vial of 7ml (0.5M sulphuric acid).
9. Specimen Diluent: 1 bottle of 20ml
10. Plate sealers: 5 pieces
11. Instruction manual: 1 copy.

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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℃.
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 450 nm and 630nm filter.
9. Gloves

Storage

1. If kept at 2 to 8℃, all the test reagents are stable until the expiry date printed on the kit.
2. Store wash solution concentrate at 2 to 8℃. If wash solution concentrate has been stored for a long time, it may be appear turbid. Performance will not be affected.
3. When the aluminum bag has been opened, the unused strips can be safely stored at 2-8℃ in the sealable plastic pouch along with the silica gel placed inside for about two weeks.

Specimen Collection And Handling

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℃ 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 Steps

1. Open the aluminum bag and take out 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). During the test, the strips must stay in the microplate.
2. Pipette 100µl of specimen diluent to the wells (leave 5 wells for controls and blank). Pipette 100µl of positive control into each of the two wells, and 100µl of negative control into each of the two wells, and pipette 100µl specimen diluent into the remaining well as a blank.
3. Using pipette, introduce 10µl of specimen to the assigned wells. (Do not add specimen to the blank well).
4. Seal and incubate for 60 minutes at 37℃.
5. Wash the micro-plate with wash solution for 5 times. (300ml/well/wash) and blot dries by pressing plate onto absorbent tissue.
6. Add 100µl of conjugate to all wells of the microplate.
7. Seal and incubate for 30 minutes at 37℃.
8. Repeat the wash procedure as step 5.
9. Pipette 50µl of Chromogen A into each well (including the blank well).

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10. Pipette 50μl of Chromogen B into each well (including the blank well).
11. Cover the plate with a fresh plate sealer. Incubate at 37℃ for 30 minutes in an incubator.
12. Stop the reaction by adding 50μl of stopping solution to each well (including the blank well) and mixing completely.
13. 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 P x 10% + N
If P is greater than or equal 2.500, let P equal 2.500.

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<0.050 and P>0. 600.
Example: P=1.960 N=0.012
Cutoff Value = P x 10% + N= 1.9600 x 10% + 0.012 = 0.208

Interpretation of Results

1. Specimens with absorbance values less than the cutoff value are considered not reactive by the criteria of this immunoassay, and may be considered negative for antibodies to HCV. Further testing is not required.
2. Specimens with absorbance values greater than or equal to the cutoff are considered reactive. These specimens (using the original sample) should be re-tested in duplicate before final confirmation of the result.
3. Initially reactive specimens, which do not react in either of the duplicate, repeat tests are considered negative for antibodies to HCV. Further testing is not required.
4. Specimens which have been found repeatable reactive are interpreted to be positive for the presence of antibodies to HCV. In most settings it is appropriate to investigate repeatable reactive specimens by additional more specific test.

Precautions

Caution:

Handle assay specimens and controls as if capable of transmitting an infectious agent. 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. Wash hands thoroughly afterwards.
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℃ or above.

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.

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3. The positive control and negative control should not be diluted.
4. The sample diluent will change colors when serum or plasma added.
5. All vials and bottles used for preparing Chromogen must be cleaned thoroughly and finally rinsed with distilled water.
6. To avoid contamination, do not touch the edges of the wells with the pipette tips when adding sample, conjugate or substrate.
7. To avoid contamination, do not touch the top of the strips with your fingers.
8. All pipetting steps should be performed with the utmost care and accuracy.
9. 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.
10. 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.

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