

HCV Ab ELISA Kit

Enzyme immunoassay for the detection of antibodies against Human Hepatitis Virus Type C (HCV)

INTRODUCTION

Hepatitis C virus (HCV), which was formerly described as the parenterally transmitted form of non-A, non-B hepatitis (NANBH)¹, becomes a chronic disease in 50% of the cases.² HCV can also be transmitted through intravenous drug abuse, sexual, and household contact.³ Hepatitis C virus is a single stranded RNA virus with some structural relations to the flavivirus family. Nucleic acid sequences of HCV cDNA clones provided the basis for the construction of recombinant peptides representing putative hepatitis C virus proteins.^{4,5} Anti-hepatitis C virus antibody screening of blood using synthetic or recombinant proteins, helped to identify apparently healthy blood donors with anti-HCV antibodies who otherwise might have transmitted the virus.⁶

This is an enzyme linked immunosorbent assay using recombinant proteins derived from core regions of HCV virus to detect the presence of HCV antibodies in human sera.

PRINCIPLE OF THE ASSAY

Multiple epitopes of HCV proteins (Core, NS3, NS4 and NS5) are bound to the microtiter wells. When antibodies to HCV are present in the test sample, they react with recombinant proteins and attach to the solid-phase. Non-reactive antibodies are removed with the wash buffer. Human IgGs bound to the antigen are reacted with goat-anti-human IgG peroxidase conjugate and visualized by subsequent reactions with a chromogenic substrate. Positive sample generates a medium to dark blue color. No color or very pale blue color indicates a negative reaction. The intensity of the reaction is photometrically quantitated.

REAGENTS

Materials provided with the kits:

1. HCV Plate: 5 microplate with 96 wells coated with HCV recombinant proteins.
2. Specimen Diluent: One bottle of 60 ml chemically defined solution containing proteins, Tween 20, and sodium azide in phosphate buffer
3. Positive Control: 2ml
4. Negative Control: 2ml
5. Enzyme Conjugate: One bottle of 60 ml Goat anti-human-IgG HRP Conjugate.
6. Substrate Solution A: 30 ml, Hydrogen Peroxide
7. Substrate Solution B: 30 ml, TMB.
8. Concentration Washing Solution (20X): One bottle (100mlX2) of phosphate buffered saline solution with Tween 20. The buffer should be diluted 20 times with distilled water before use.
9. Stop Solution: 30 ml of 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.

PRECAUTION FOR USERS

All human source material used in the preparation of this product was found to be negative for the presence of HIV-1/HIV-2 antibodies, as well as for the hepatitis B surface antigen, using a commercial licensed method. Nevertheless, because no test method can offer complete assurance of the absence of infectious agents, this product should be handled with caution.

1. Avoid contact of reagents with the eyes and skin. If that occurs, wash thoroughly with water.
2. Wear gloves.
3. Do not pipette by mouth.
4. Do not smoke.
5. Dispose all used materials in a suitable biohazardous waste container. Remains of samples, controls, aspirated reagents and pipette tips should be collected in a container for this purpose and autoclaved 1-hour at 121°C or treated with 10% sodium hypochlorite (final concentration) for 30 min before disposal. (Remains containing acid must be neutralised prior addition of sodium hypochlorite).

6. Adjust washer to the plate used (flat bottom) in order to wash properly.
7. Do not mix reagents from different lots.
8. Do not use reagents after expiration date.
9. Extreme care should be taken to avoid microbial contamination and cross contamination of reagents.
10. Use a new pipette tip for each specimen and each reagent.
11. Soaps and/or oxidising agents remaining in containers used for the substrate-TMB solution can interfere with the reaction.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum or plasma can be used in this test. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Specimen with extensive particulate should be clarified by centrifugation prior to use. Specimen frozen at -20°C or colder may be used. Avoid repeated freeze thaw.

STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

ASSAY PROCEDURE

1. Dispense 100µl of specimen diluent into individual test wells.
2. Dispense 10µl positive control and negative control duplicate into individual wells.
3. Add 10µl of each test sample into duplicate test wells; vortex to mix.
4. Incubate for 30 minutes at 37°C
5. Wash each well 5 times by filling each well with diluted 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 100µl of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 minutes. **Do not add Enzyme Conjugate to the blank well.**
7. Incubate for 20 minutes at 37°C
8. Wash the plate 5 times as step 6.
9. Add one drop (50µl) of Substrate Solution A (HRP-substrate) to each well, then add one drop (50µl) of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 10 minutes. .
10. Add one drop (50µl) of Stop Solution to each well to stop the color reaction. Read O.D. at 450 nm with an EIA reader.

RESULT INTERPRETATION

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells, The positive control OD value should be ≥ 0.8 , the negative control should be ≤ 0.10):

Cut-off Calculations:

Take average OD values of Negative control and add 0.15:

$$1 \times \text{NC} + 0.15 = \text{Cut-off.}$$

Positive OD reading: \geq Cut-off value

Negative OD reading: $<$ Cut-off value

LIMITATIONS OF THE ASSAY

1. As the 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 entire content of wells completely before adding the washing solution.
2. 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.
3. Samples with positive or equivocal result must be reanalysed in duplicate. If both retest values are lower than the cut-off, the final interpretation of the test is negative for HCV antibodies. If the result is repeatedly positive or equivocal, the sample should be further investigated with other methods.
4. Optimal assay performance requires strict adherence to the assay procedure described. Deviation from the procedure may lead to aberrant results.
5. A negative result does not exclude the possibility of exposure or infection with HCV

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In vitro diagnostic device



Consult instructions for use



Keep dry



Temperature limitation



Use by



Lot code



Catalogue number



Contains sufficient for <n> tests



Manufacturer



Do not use if package damaged

Manufacturer:

Biocare Diagnostics Ltd.
6F, Building B, 108 Xinghua Road
Xiangzhou, Zhuhai, China 519000
Tel: +86-756-8238560
E-mail: info@ivdbiocare.com
Website: www.ivdbiocare.com

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