AccuDiag™ HCV Ab ELISA Kit

REF 1707-P1

IVD See External Label 2°C-8°C Σ 96 Tests

HCV Ab ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Solid Phase Indirect ELISA
Detection Range	Qualitative: Positive & Negative Control
Sample	10 µl
Incubation Time	60 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation, Inc. (DAI) HCV Ab ELISA is an enzyme-linked immunosorbent assay (ELISA) for the qualitative determination of antibody to Hepatitis C Virus in human serum or plasma.

SIGNIFICANCE AND SUMMARY

The term non-A, non-B hepatitis (NANBH) has been used to describe hepatitis in patients who do not develop antibodies to hepatitis A, hepatitis B, cytomegalovirus or Epstein-Barr virus and do not have a clinical history of other potential causes of hepatitis. Later on, HCV was identified as one of the major agent of NANBH, which is associated with post-transfusion hepatitis. The cloning of HCV from the plasma of a chronic NANBH chimpanzee led to the development of recombinant antigens, which were used in immunoassay system to detect HCV.

Individual blood or plasma donors or patients may be tested for the antibody to HCV. The presence of these antibodies indicates that the individual (donor or patient) has been infected with HCV, may harbor infectious HCV, and may be capable of transmitting NANBH.

ASSAY PRINCIPLE

The wells of the polystyrene microplate strips are coated with purified recombinant HCV antigens, which correspond to the structural and non-structural regions of the Hepatitis C Virus. Human serum or plasma, diluted in sample diluent, are incubated in these coated wells. HCV specific antibodies, if present, will bind to the solid phase HCV antigens. The wells are thoroughly washed to remove unbound materials and an anti-human Ab labeled with horse radish peroxidase is added to the wells. This labeled antibody will bind to any antigen-antibody complexes previously formed and excess unbound labeled antibodies are removed by washing. Substrate solution containing hydrogen peroxide and 3,3', 5,5'-tetramethylbenzidine (TMB) is then added to each well. The presence of specific antibodies is indicated by the presence of a blue color after substrate addition. Reaction is terminated by addition of sulphuric acid. The intensity of the color is measured spectrophotometrically at 450nm and is proportional to the amount of antibodies present in the specimen.

MATERIALS AND COMPONENTS

Materials provided with the test kit

1. **Microplate 1 plate (96 wells).**
   12 strips per plate, each with 8 wells coated with a mixture of purified recombinant HCV core, NS3, NS4, and NS5 antigens.
2. **Positive Control 1 vial (0.5ml).**
   Inactivated Human serum containing antibodies to HCV antigens. Preservative: Bronidox (2ml/L).
3. **Negative Control 1 vial (0.5ml).**
   Inactivated Human serum without antibodies to HCV antigens. Preservative: Bronidox (2ml/L).
4. **Conjugate 1 vial (6ml).**
   The solution contains HRP-labeled anti-human IgG (goat), with PBS (0.01mol/L) and stabilizing proteins, ready to use.
5. **Sample Diluent 1 vial (11ml).**
   Contains stabilizing proteins and detergent, Preservative: Bronidox (2ml/L).
6. **Wash Buffer (30x) 1 vial (25ml).**
   Diluted 25-fold in distilled water as described in section of Preparation of Reagent.
7. **TMB Color reagent 1 vial (11ml).**
8. **Stop Solution 1 vial (11ml).**
   2N sulfuric acid.
9. **Instruction Manual 1 booklet**

Materials required but not provided

1. Disposable absorbent: benchtop paper and paper towels.
2. Graduated pipettes: 10 ml.
3. Multichannel pipettor capable of delivering 10µl, 50µl, and 100µl.
4. Disposable pipette tips.
5. Reagent reservoirs (troughs) with a capacity of 25ml.
6. Deionized or distilled water, reagent grade quality.
7. Flasks: 500ml, 1 liter.

8. ELISA Microplate Washer. Alternatively, Washing can be performed manually.
9. A 37°C incubator.
10. ELISA Microplate Reader.
11. Sodium hypochlorite (5%) solution or liquid household bleach.

STORAGE

Store the kit and its components at 2~8°C when not in use.

HANDLING PRECAUTIONS

1. Optimal assay performance requires **strict adherence** to the assay procedure described in this Instruction Manual. Deviations from the procedure may lead to aberrant results.
2. **Do not substitute reagents from one kit lot to another.** Controls, conjugate and microplates are matched for optimal performance. Use only the reagents supplied with the kit.
3. During 37°C incubation, evaporation must be prevented.

PREPARATION OF REAGENT

1. All reagents should be allowed to reach room temperature (25±3°C) before use.
2. Dilute 1 volume of **wash buffer (30x)** with 29 volumes of distilled water before use.

ASSAY PROCEDURE

1. Add 100µl of **sample diluent** to wells A1 and B1 as blank control.
2. Add 100µl of **negative control** to wells C1 and D1, and add 100µl of **positive control** to wells E1 and F1.
3. Dispense 100µl of **sample diluent** in other wells.
4. Add 10µl of specimen into the assigned well, starting at G1.
5. Carefully cover microplate with a **plate cover** to prevent evaporation during incubation.
6. Incubate for 30 minutes at 37°C.
7. Wash the microplate with **diluted wash buffer.**
   A. ELISA Microplate Washer - Wash 5 times with 300 µl/well/wash.
   B. Manual Microplate Washer-Aspirate completely the contents of all wells by lowering the aspirator tip gently to the bottom of each well. **Be careful not to scratch the inside of the well surface.** Fill the entire plate with 300 µl/well, then aspirate in the same order. Perform this cycle 5 times.
8. Blot dry by inverting the microplate and tapping firmly onto absorbent paper. All residual plate wash buffer should be blotted dry.
9. Add 50µl of **conjugate** to each well **(except for the blank control).** Apply another **plate cover.**
10. Incubate for 20 minutes at 37°C.
11. Repeat the wash procedure as in Step 7 and Step 8.
12. Add 100µl of **TMB color reagent** to each well.
13. Incubate for 10 minutes at 37°C, away from direct or intense light.
14. Stop the reaction by adding 50µl (or one drop) of **stop solution** to each well.
15. Determine the absorbance for each well at 450nm with a microplate reader.

SAFETY PRECAUTIONS AND DISPOSAL

1. **Handle assay specimens, Positive and Negative controls as potentially infectious agents.** Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in biohazard waste-bags. Wash hands thoroughly afterwards.
2. Autoclave all used and contaminated materials at 121°C, 15 psi for 30 minutes before disposal. Alternatively, decontaminate materials in 5% sodium hypochlorite solution for 30-60 minutes before disposal in biohazard waste-bags.
3. Wipe any spills promptly with 1% sodium hypochlorite solution.
4. Sulphuric acid can cause severe burns. **Avoid contact with the reagent.** If the reagent came into contact with skin, wash thoroughly with water.

QUALITY CONTROL

1. The **Blank Control, Negative Control and Positive Control** should be assayed **(ONE WELL AT LEAST)** in duplicate on each plate with each run of specimens.
2. Blank value must have an absorbance of < 0.100.
3. **Negative Control** values must have an absorbance of ≤ 0.100 after subtracting the Blank.
4. Each of the 2 **positive Control** values must have absorbance ≥ 0.600 after subtracting the Blank.
5. For the assay to be valid, the difference between the mean absorbance of the **Positive Control** and the **Negative Control (PCx-NCx)** would be 0.500 or greater. If not, technique may be suspected and the assay must be repeated. If **PCx-NCx** is consistently low, deterioration of reagents may be suspected.

RESULTS

Each microplate must be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. **The absorbance values of the blank control must be subtracted from both the controls' and the specimens' absorbance values before interpretation of results.**

The presence or absence of IgG antibodies specific for HCV is determined by relating the absorbance of the specimens to the CUT-OFF Value of the plate. The CUT-OFF Value for HCV-Ab ELISA is calculated as 0.15 + the Mean Absorbance of the Negative Control (If the mean OD<0.05, CUT-OFF Value =0.15+0 .05=0.200).

CALCULATION OF RESULTS

1. Calculation of Negative Control Mean Absorbance (NCx).
   Individual Negative Control values should be less than or equal to 0.100 unit. All individual Negative Control values must meet the criteria or the assay is invalid and must be repeated.
2. Calculation of Positive Control Mean Absorbance (PCx).
   Individual Positive Control values must be greater than or equal to 0.600 units. If one Positive Control value does not meet either of the above criteria, the assay is invalid and must be repeated.
3. Calculation of the difference between PCx and NCx.
   For the assay to be valid, the PCx-NCx value should be 0.500 or greater. If not, improper technique or deterioration of reagents may be suspected and the assay should be repeated.
4. Calculation of CUT-OFF value.

CUT-OFF Value= 0.15+NCx (If NCx< 0.05, CUT-OFF Value =0.200.

INTERPRETATION OF RESULTS

1. The HCV-Ab ELISA considers specimens with absorbance values less than CUT-OFF value Negative.
2. Specimens with absorbance values **greater than or equal to** the CUT-OFF value are considered initially positive by the criteria of the HCV-Ab ELISA and should be retested in duplicate before interpretation.
3. Specimens found positive on retesting are interpreted to be **repeatedly positive** for antibodies to HCV by the criteria of the HCV-Ab ELISA.
4. Initially positive specimens which are Negative on retesting are considered negative by the criteria of the HCV-Ab ELISA.

LIMITATIONS OF THE PROCEDURE

Repeatedly positive results from the HCV-Ab ELISA is presumptive evidence of antibodies to HCV in the specimen. A non-reactive result from the HCV-Ab ELISA indicates the likely absence of detectable antibodies to HCV in the specimen. A negative result does not exclude the possibility of exposure to or infection with HCV.

Falsely positive results can be suspected with a test kit of this nature. The proportion of false positives will depend on the sensitivity and the specificity of the test kit. For most screening assays, the higher the prevalence of antibody in a population, the lower the proportion of falsely positive samples.

REFERENCES

1. Alter HJ, Pullcell RH, Holland PV, et al. Transmissible agent in non-A, non-B hepatitis. Lancet *i* :459-463, 1978.
2. Dienstag JL. Non-A, non-8 viral hepatitis. J. Virol. Methods 10:307-319, 1985.
3. Choo QL, Kuo G, Weiner AJ, et al. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 244:359-362, 1989.
4. Kuo G, Choo QL, Alter HJ, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-8 hepatitis. Science 244:362-364, 1989.
5. Alter HJ, Colemen PJ, Alexander WJ, et al. Importance of heterosexual activity in the transmission of hepatitis B and non-A, non-8 hepatitis. JAMA 262:1201-1205, 1989.
6. Van der Polel CL, Lelie PN, Choo QL, et al. Anti-hepatitis C antibodies and non-A, non-8 post-transfusion hepatitis in the Netherlands. Lancet *ii*:324, 1989.
7. Kuhnl P, Se dl S, Stangel W, et al. Antibody to hepatitis C in German blood donors. Lancet ii :324-325, 1989.
8. Van der Poel CM, Reesink HW, Schaasberg W, et al. Infectivity of blood seropositive for hepatitis C virus antibodies. Lancet 335:558-560, 1990.

MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116
Woodland Hills, California 91367 USA

Date Adopted	**2022-09**
Brand Name	**AccuDiag™**
REF 1707-P1	**AccuDiag™ - HCV Ab ELISA**
Revision Date: 2022-08	