

**AccuDiag™
HCV ELISA**

REF 1707-12



Test	HCV Ab ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Solid Phase Indirect ELISA
Detection Range	Qualitative: Positive & Negative Control
Sample	10ul
Total Time	~ 75 min
Shelf Life	12 Months from the manufacturing date

96-well ELISA kit for the Qualitative Detection of Antibodies to Hepatitis virus C in Human Serum, plasma, Store at 2 - 8°C Upon Receipt

INTENDED USE

The Diagnostic Automation, Inc. (DAI) HCV ELISA kit is to be used for the qualitative detection of IgG antibodies to Hepatitis C virus (HCV) in human serum and plasma. It is intended for professional use only.

SUMMARY AND EXPLANATION

Hepatitis C Virus (HCV) is a small, enveloped, positive-sense, single-stranded RNA Virus(1). HCV is now known to be the major cause of the blood transmitted non-A, non-B hepatitis(2). Antibodies to HCV are detectable about 45 days after exposed to HCV, and are found in over 80% of patients with well-documented non-A, non-B hepatitis. Therefore, detection of HCV antibodies in the serum or plasma is useful in the determination of HCV exposure and in the diagnosis of Hepatitis C(3,4).

The HCV ELISA kit is a latest generation of solid phase enzyme linked immunoassay which specifically detects antibodies to HCV in human serum or plasma. The test is highly sensitive and specific.

TEST PRINCIPLE

The HCV ELISA kit utilizes HRP conjugated anti-human IgG and multiple recombinant HCV antigens to detect HCV antibodies qualitatively and selectively in serum or plasma. The test microwells are coated with multiple recombinant antigens including structure and non-structure proteins. The HCV antibodies, if present in the test sample, bind to the recombinant antigens immobilized on the microwell surface. The bound antibodies then react with horseradish peroxidase enzyme (HRP) conjugated anti-human IgG, forming an antigen-HCV antibody- anti-antibody enzyme complex on the microwell surface. Upon addition of TMB substrate, the HRP enzyme converts bound complex into a color signal. The intensity of the color is directly proportional to the concentration of HCV antibodies in the samples.

SPECIMEN COLLECTION AND PREPARATION

- Serum should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
- This kit is designed for use with serum sample without additives only.
- If a specimen is not tested immediately, refrigerated at 2°-8°C. If storage period greater than three days are anticipated, the specimen should be frozen (-20°C). Avoid repeated freezing-thawing of samples. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.

Do not use serum samples demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

MATERIALS AND COMPONENTS

Materials and reagents provided with the kit

1. Twelve (12) 1x8-well strips coated with recombinant HCV antigens. The strip is packaged in a strip holder and sealed in the foiled envelope with desiccant.
2. Sample diluent 11 ml
3. HCV antibody negative control 0.5 ml
4. HCV antibody positive control (Deactivated) 0.5 ml
5. Anti-human IgG- HRP conjugates 6 ml
6. Wash Buffer (30x concentrate). 25 ml
7. TMB substrate 11 ml
8. Stop solution 10 ml
9. Product insert 1 set

Materials and reagents required but not provided in the kit

- . Pipette capable of delivering 5-50 ul volumes with a precision better than 1.5%.
- 2. Dispenser(s) for repetitive deliveries of 50ul and 200ul ml volumes with a precision better than 1.5%.
- 3. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable
- 4. Absorbent paper for blotting the microplate wells.
- 5. Parafilm or other adhesive film sealant for sealing plate.
- 6. Timer.

PRECAUTIONS

TO BE USED ONLY FROM QUALIFIED PROFESSIONALS

The test kit does not contain any viable infectious agents. However all patient samples must be considered as potentially infectious.

- Do not ingest the reagents. Avoid contact with eyes, skin and mucose. Wear protective clothing and disposable gloves. Do not allow smoking or eating where antigen containing materials are being handled.
- Preclude any pipetting by mouth.
- Handle all patient samples and test components as though capable of transmitting infection. Clean spills thoroughly using an appropriate intermediate-to high level disinfectant. Decontaminate and dispose of specimens and all potentially contaminated materials as if they contain infectious agents.
- Avoid splashing or aerosol formation.
- Avoid contacting Substrate Reagent or Stop solution with the skin or other mucose membranes. In case of contact, wash thoroughly with water.

PRODECURE NOTES

- Reliable and reproducible results will be obtained when The assay procedure is carried out with a complete understanding of the package insert instruction.
- Do not splash liquid while rocking or shaking the wells.
- The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.
- Avoid strong light during color development. In the beginning of each incubation and after adding Stopping Solution, gently rocking the microwells to ensure thorough mixing. Avoid the formation of air bubbles as which results in inaccurate absorbance values.

ASSAY PROCEDURE

A: Preparation

1. Bring all reagents, standards, controls to room temperature (18-25°C).
2. Dilute concentrated Washing Buffer 30 fold with distilled water. Add 725 ml of distill water to 25 ml concentrated Washing Buffer and mix well. Warm up the concentrated washing buffer if precipitants appear.
3. Mix each reagent before adding to the test wells.

B: Assaying

1. Remove the desired number of microwells from the bag.
2. Set up duplicate wells for negative and positive control, respectively. Add 100 ul of **prediluted** negative and positive control to the designated wells.
3. Add 100 ul (2 drops) of sample diluent to the test wells, then transfer 10 ul of test samples to the test wells. Gently rock the wells for twenty second, then cover the wells.
4. Incubate the wells at 37°C for 30 minutes.
5. Carefully remove the incubation mixture by emptying the solution into a waste container. Fill each well with diluted wash buffer and shake gently for 20-30 second. Discard the wash solution completely by inverting and tapping the plate on absorbent paper. Repeat above procedure 4 more times.
6. Add 50 ul (1 drops) of anti-human IgG-HRP enzyme into each well, cover it, and incubate at 37°C for 20 minutes.
7. Wash the plate 5 times as step 6 described.
8. Add 100 ul (2 drops) of TMB substrate into each well.
9. Incubate at 37°C in the dark for 10 minutes.
10. Stop the reaction by adding 50 ul-100 ul (1-2 drops) of stop solution to each well. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
11. Set the microplate reader wavelength at 450 nm and measure the absorbance of each well.

INTERPRETATIONS OF THE RESULTS

A: Set up the cutoff value

The cutoff value = 0.15 + N

N: Mean OD of the negative control. Use 0.05 for calculation if N value is less than 0.05.

B. Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the Cutoff Value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cutoff Value}}$$

C. Interpretations

Specimen OD ratio
 Negative < 1.00
 Positive ≥ 1.00

The negative result indicates that there is no detectable anti HCV antibodies in the specimen.

The specimen with a positive result should be tested duplicate again and confirmed with Western blot or other tests.

D. Quality Control

The mean OD value of the positive controls - -the mean OD value of the negative controls should be greater than 0.300.

If not, the test should be considered invalid. Please check the procedure and repeat the assay.

STORAGE

- Test components are stable up to their expiration data when stored at 2°-8°C. Do not freeze.
- Return all reagents requiring refrigeration immediately after use. Reseal the microwell bag immediately after removing the desired number of wells. Keep the desiccant in the bag at all times during storage.
- Do not mix or use components from the kits with different lot numbers. Do not use reagents after their expiration date.



LIMITATIONS

- The HCV ELISA kit is limited to the detection of HCV antibodies in human serum, plasma.
- The test is a qualitative screening assay and is not for determining quantitative concentration of HCV virus antibodies.
- A negative result does not rule out HCV infection because the antibodies to HCV may be absent at the time the specimen is taken or may not be present in sufficient quality to be detected at early stage of infection.

- As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after evaluation of all clinical and laboratory findings.

REFERENCES

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<p>ISO 13485 ISO 9001</p>  <p> Diagnostic Automation/ Cortez Diagnostics, Inc. 21250 Califa Street, Suite 102 and 116 Woodland Hills, California 91367 USA</p>	
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