Hepatitis Virus C (HCV) IgG ELISA Kit

- **96-well ELISA kit for the Qualitative Detection of IgG Antibodies to Hepatitis C virus in Human Serum, plasma**
- **For Export Only, Not for Re-sale in the USA.**
- **Not for Internal or External Use in Human or Animal.**
- **Store at 2 - 8ºC Upon Receiving.**

INTENDED USE

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

INTRODUCTION

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 IgG 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.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

1. 8 x 12-well strips coated with recombinant HCV antigens (*Cat#E0510W)*
2. 11 ml Sample diluent (*Cat#E0510SD)*
3. 1 ml HCV antibody negative control (*Cat#E0510N) Ready to use*
4. 1 ml HCV antibody positive control (*Cat#E0510P) Ready to use*
5. 11 ml HRP-anti human IgG conjugates (*Cat#E0510H)*
6. 20 ml Wash Buffer (30xconcentrate) (*Cat#WE3000)*
7. 11 ml TMB substrate (*Cat#TME2000*)
8. 6 ml Stop solution (*Cat#SE1000)*
9. 1 set Product insert
10. 2 sets ELISA Working Sheet

Materials and reagents required but not provided in the kit

1. 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 0-3 OD or greater at 450 nmwavelength is acceptable
4. Absorbent paper for blotting the microplate wells.
5. Parafilm or other adhesive film sealant for sealing plate.
6. Timer.

STORAGE AND STABILITY

- Test components are stable up to their expiration data when stored at 2º-8ºC. Do not freeze.
- Return all reagents requiring refrigeration immediately after use. Reseal the microwells after removing the desired number of wells.
- Do not mix or use components from the kits with different lot numbers. Do not use reagents after their expiration date.

WARNING AND PRECAUTIONS

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 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.

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 2º-8ºC. If storage period greater than three days are anticipated, the specimen should be frozen (-20ºC). 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 lipermia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PROCEDURE 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, controls to room temperature (18-26˚C).
2. Dilute concentrated Washing Buffer 30 fold with distilled water. Add 580 ml of distill water to 20 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.
4. Determine the number of microwells needed and mark on the ELISA Working Sheet with the appropriate information. Positive, and Negative Controls require be run in duplicate to ensure accuracy.

B: Assaying

1. Remove the desired number of strips and re-seal un-used strips.
2. Add **100 ul** of positive, negative control into appropriate 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. Leave the blank well alone.
   To ensure better precision, use pipette to handle solution.
4. Incubate the wells at **37°C for 30 minutes**.
5. Carefully remove the incubation mixture by empting 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 and tapping the plate on absorbent paper. Repeat above procedure 4 more times.
6. Add **100 ul (or 2 drops)** of HRP- anti-human IgG conjugates into each well, cover it, and incubate at **37°C for 20 minutes**.
7. Wash the plate 5 times as step 5 described.
8. Add **100 ul (or 2 drops)** of TMB substrate into each well.
9. Incubate at **room temperature (18-26˚C) in dark** for **10 minutes**.
10. Stop the reaction by adding **50 ul (1 drop)** of stop buffer 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 against the blank well within 15 minutes after adding Stop Solution. A filter of 620 -690 nm can be used as a reference wavelength to optimize the assay result.

INTERPRETATION OF 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 of the cutoff value if 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 should be ≥ 0.50.
The mean OD value of the negative controls should be ≤ 0.10.

Check the procedure and repeat assay if above conditions are not met.

LIMITATION OF THE TEST

1. The HCV IgG ELISA kit is limited to the detection of HCV antibodies in human serum or plasma.
2. The test is a qualitative screening assay and is not for determining quantitative concentration of HCV virus antibodies.
3. 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.
4. 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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3. Van der Poel, C. L., H.T.M. Cuypers, H.W. Reesink, and P.N.Lelie. Confirmation of hepatitis C Virus infection by new four-antigen recombinant immunoblot assay. Lancet 1991; 337:317
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