Hepatitis C Virus (HCV) Antibody ELISA Test Kit (Serum/Plasma)

Package Insert

For in vitro diagnostic and professional use only.

PRODUCT NAME

Hepatitis C Virus (HCV) Antibody ELISA Test Kit (Serum/Plasma)

INTENDED USE

This kit is intended for the qualitative detection of hepatitis C virus (HCV) antibody in human serum/plasma, which is based on Enzyme Linked Immunosorbent method. The kit is suitable for clinical screening and diagnosis of HCV infection in serum/plasma.

TEST PRINCIPLE

This kit uses indirect ELISA principle to detect HCV antibody. Purified HCV antigen is pre-coated on the microplate, the enzyme-labeled anti- HCV complex will combine with HCV antibody in human serum/plasma.

HCV recombinant antigen is adsorbed in solid phase to the polystyrene reaction microplate. If there is HCV antibody in test sample, it binds to HCV antigen coated in microplate, forms antigen-antibody complex, and then binds to the enzyme labeled anti-antibody and forms antigen-antibody-antibody complex on surface of the microplate, and display blue color in corresponding well via the action of substrate. Therefore, it can detect the HCV specifically in human serum/plasma.

COMPONENTS

Materials provided with the kit:

Components	96T/Box		480T/Box	
Coated Plate	1 bag	96 wells	5 bags	96 wells
Enzyme Conjugate	1 vial	8 mL	5 vials	8mL
Wash Buffer (40X)	1 vial	20 mL	5 vials	20 mL
Sample Diluent	1 vial	12 mL	5 vials	12 mL
Substrate A	1 vial	7 mL	5 vials	7 mL
Substrate B	1 vial	7 mL	5 vials	7 mL
Stop Solution	1 vial	6 mL	5 vials	6 mL
Negative Control	1 vial	1 mL	5 vials	1 mL
Positive Control	1 vial	1 mL	5 vials	1 mL
Closure Plate Membrane	3 sheets		15 sheets	

Note: different batches of reagent kit, and different components cannot be exchanged for use. Once open, stable for 3 months at 2-8°C.

SAMPLE REQUIREMENTS

1. **Specimen Collection:** No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum/plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum must be separated from the clot as early as possible to avoid hemolysis of the RBC. Care should be taken to ensure that the serum/plasma specimens are clear and not contaminated by microorganisms.

2. ***Highly lipaemic, icteric, or hemolytic specimens should not be used*** as they can give false results in the assay. ***Do not heat inactivate specimens***. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.

3. HCV ELISA is intended ONLY for testing of individual serum/plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

4. Transportation and Storage: Store specimens at 2-8°C. Specimens not required for assaying within 3 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

TEST PROCEDURE

1. All reagents should be allowed to reach room temperature for at least 15 minutes before use.

2. Dilute the wash buffer at the rate of 1:40 dilution with distilled water before use.

3. The sample should be corresponding to the number of microplate, each plate should be provided with negative control 3 wells, positive control 2 wells and blank control 1 well. (If detect with dual wavelength detection, setting no blank control well is allowed).

Note: Use a separate disposal pipette tip for each specimen, Negative and Positive Control to avoid cross contamination.

4. Add 100µL Sample Diluent in the corresponding well (Do not add in the blank well, negative control wells and positive control wells).

5. Add 10µL sample in the corresponding well, mix thoroughly by using the pipette, add 100µL negative control and positive control to negative control wells and positive control wells (Do not add in the blank well) respectively.

6. Shake for 30 seconds with an oscillator (This step is very important). Incubate at 37°C for 60 minutes with the sealing plate membrane sealing the plate.

7. At the end of the incubation, remove and discard the plate cover. Take out, add wash buffer to each well for 20 seconds. Repeat 5 times. After the final washing cycle, turn the plate over onto blotting paper or clean towel, and tap it to remove any remainders.

8. Respectively adding Enzyme Conjugate 50µL (Do not add in the blank well). Mix gently by shaking. Incubate at 37°C for 30 minutes with the sealing plate membrane sealing the plate.

9. Repeat the wash step for 5 times as in step 7.

10. Add Substrate A (50µL) and Substrate B (50µL) (Do not add in the blank well). Mix gently by shaking. Incubate at 37°C for 30 minutes with the sealing plate membrane sealing the plate.

11. Add 50µL Stop Solution to each well (Do not add in the blank well). Mix gently by shaking, read the absorbance within 10 minutes after stopping the reaction. Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630 nm. Set no blank wells is allowed if use dual wavelength to detect. Calculate the Cut-off value and evaluate the results.

INTERPRETATION OF RESULTS

Colorimetry: Read the sample's optical density (OD) at 450nm with a microplate reader.

Mean negative control OD value ≤ 0.1 and positive control OD value ≥ 0.8, the test is valid, otherwise the test is invalid.

Cut-Off value (C.O.) = Mean negative control OD value + 0.12

Positive Results: Sample OD value/C.O. ≥ 1.0

Specimens giving an absorbance equal to or greater than the Cut-Off value are considered initially reactive, which indicates that HCV antibody has probably been detected using HCV ELISA. All initially reactive specimens should be retested in duplicates using HCV ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for HCV antibody with HCV ELISA.

Negative Results: Sample OD value/C.O. < 1.0

Specimens giving absorbance less than the Cut-Off value are negative for this assay, which indicates that no HCV antibody has been detected with HCV ELISA, therefore the patient is probably not infected with HCV and the blood unit do not contain HCV antibody and could be transfused in the case that other infectious diseases markers are also absent.

Borderline: Sample OD value/C.O.= 0.9-1.1

Specimens with OD value to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicate is required to confirm the results.

Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system (e.g. PCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

LIMITATIONS

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. The reagent is a qualitative reagent, and cannot be used as a quantitative reagent.
3. This reagent is only used for the detection of human serum/plasma samples.

PERFORMANCE CHARACTERISTICS

1. Negative Specificity: The coincidence rate should not lower than 29/30 when detecting negative quality control samples with ELISA kits of anti-HCV.
2. Positive Specificity: The coincidence rate should not lower than 29/30 when detecting positive quality control samples with ELISA kits of anti-HCV.
3. Limit of Detection: L1 should detect positive, L2 should detect positive, L3 can detect positive or negative, L4 should detect negative when detecting with 4 HCV limit quality control samples with the ELISA kits of anti-HCV.
4. Precision: Intra-assay: CV%≤15%; Precision between batches: CV%≤20%.

ATTENTIONS

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. Allow the reagents and specimens to reach room temperature before use. Shake reagent gently before use.
4. Concentrated washing liquid will produce crystal at room temperature, should be diluted completely before use.
5. Please put the unused plate back into the bag, and store at 2-8°C.
6. Operate strictly according to the instruction, control of reaction time and temperature strictly.
7. Never reuse microplate sealing membrane. If the external of the microplate contact with water when warm bath, results will be better.
8. Use sufficient volume of washing liquid in the washing steps. Fail to do so may cause color deepening.
9. Negative results of reagent do not rule out the possibility of HCV infection. Positive results must be combined with clinical information for analysis.
10. All the reagents were treated by inactivation, but still should be regarded as potentially infectious. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.

STORAGE AND EXPIRY

1. Store at 2-8°C.DO NOT FREEZE. Valid for 18 months.
2. Once open, stable for 3 months at 2-8°C. Other liquid components have the same validity period with the reagent box.

BIBLIOGRAPHY

[1] Pharmacopoeia of the People's Republic of China.
[2] Requirements for Biologics of the People's Republic of China.

INSTRUCTIONS OF SYMBOL

	Consult instructions for use		Keep dry
	Temperature limit		Batch code
	Do not re-use		In vitro diagnostic medical device
	Manufacturer		Date of manufacture
	Use-by date		Contains sufficient for <n> tests
	Keep away from sunlight		

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