(For Research Use Only. Not For Use In Diagnostic Procedures!)

HCV (Hepatitis C Virus) ELISA Kit

(Do not mix reagents of different batches and different product numbers in the kit, otherwise t he kit will not work properly)

Catalogue No.: EU2675

Size: 96T

Reactivity: Universal

Application: This immunoassay kit allows for the qualitative determination of HCV in serum, plasma, cell culture supernatant and other biological samples.

Storage: 2-8℃

Expiry Date: see kit label

Principle: Sandwich

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Size(48T)	Size(96T)	Storage
Micro ELISA Plate(Dismountable)	8×6	12 × 8	2-8℃/-20℃
HCV Positive Control	0.5ml	1ml	2-8℃
HCV negative Control	0.5ml	1ml	2-8℃
HRP- Anti-HCV Antibody （Concentrated, 100X）	30ul	60ul	2-8℃(Avoid Direct Light)
TMB substrate	5ml	10ml	2-8℃(Avoid Direct Light)
Antibody Dilution Buffer	3ml	6ml	2-8℃
Stop solution	5ml	5ml	2-8℃
WashBuffer (25X)	15ml	30ml	2-8℃
Plate Sealer	3 pieces	5 pieces	
Product Description	1 copy	1 copy	

Note: The liquid reagent bottle contains slightly more reagent than indicated on the label. Use a micropipette to measure accurately.

Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was pre coated onto the 96-well plate. Add HRP-labeled HCV antibody, add the control and samples. If the sample contains HCV , the immunocomplex “HCV antibody - HCV antigen - HRP-labeled HCV antibody ” forms during the reaction. After incubation, unbound conjugates were removed by wash buffer. TMB was catalyzed by HRP to produce a blue color product that turned yellow after adding acidic stop solution. Read the O.D. absorbance at 450nm in a microplate reader.

Precautions for Use

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don’t let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don’t reuse tips and tubes to avoid cross contamination.
9. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manuf acturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Material Required But Not Supplied

1.Microplate reader (wavelength: 450nm)

2.37℃ incubator

3.Automated plate washer

4.Precision single and multi-channel pipette and disposable tips

5.Clean tubes and Eppendorf tubes

6.Deionized or distilled water

Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20℃ for long term. Avoid multiple freeze-thaw cycles.

- Serum: Coagulate the serum at room temperature (about 1 hour). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20℃.

Note: Samples to be used within 5 days may be stored at 2-8℃, otherwise samples must be stored at -20℃ (≤1 month) or -80℃(≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Reagent Preparation and Storage

Take the Elisa kit from the fridge around 20 minutes earlier and equilibrate to room temperature(18-25℃). For repeated assays, please just take the strips and standards required for the current assay, store the rest materials according to the relevant condition.

1. Wash Buffer

Dilute 30ml (15ml for 48T) concentrated wash buffer to 750ml (375ml for 48T) wash buffer with deionized or distilled water and mix well. (The recommended resistivity of ultrapure water is 18MΩ.) Alternatively, take appropriate amount of concentrated wash buffer according to the assay requirement, then create a 25-fold dilution and mix well. Store the rest solution at 2-8℃.

Crystals formed in the concentrated wash buffer can be heated by water bath at 40℃ till complete dissolution. (Heating temperature should be below 50℃.) Mix well for the next step. It's better to use up the prepared wash buffer in one day. Store the rest buffer at 2-8℃ within 48h.

2. HRP- Anti-HCV Antibody Working Solution

The working solution should be prepared within 30min before the assay and can't be stored for a long time.

3.1. Calculate required total volume of the working solution: 50ul/well x quantity of wells. (It's better to prepare additional 100ul-200ul.)

3.2. Centrifuge for 1min at 1000xg in low speed and bring down the concentrated HRP- Anti-HCV Antibody to the bottom of tube.

3.3. Dilute the HRP- Anti-HCV Antibody with Antibody Dilution Buffer at 1/100 and mix them thoroughly. (e.g. Add 10ul concentrated HRP- Anti-HCV Antibody into 990ul Antibody Dilution Buffer.)

Detailed Assay Procedure

Step 1: Take required amount of strips. Then fix strips on the plate and number them. Set 3 negative control well, 2 Positive Controls and 1 blank wells (Don't add samples and HRP-Conjugates).

Step 2: HRP- Anti-HCV Antibody and control/sample loading: Add 50ul HRP- Anti-HCV Antibody Working Solution into each well. Then, add 50ul negative and positive control and samples in the appropriate well respectively. Immediately, gently tap the plate for 10s to ensure thorough mixing then static incubate for 60 minutes at 37°C. (When adding control or sample, the disposable tip lightly touches the liquid level. Change the disposable tips for different samples and standards.)

Step 3: Wash five times: After incubation, remove the cover and wash plate 5 times with wash buffer. Soak for 30-60 seconds. After the 5th washing, remove all wash buffers through aspiration or pouring.

Step 4: TMB Substrate: Add 90ul TMB Substrate into each well, seal the plate and static incubate at 37°C in dark within 10-20 minutes. Run the microplate reader and preheat for 15min.

Step 5: Stop: Keep the liquid in the well after staining. Add 50ul stop solution into each well. The color will turn yellow immediately. The order for adding stop solution and TMB substrate solution is the same.

Step 6: OD Measurement: Read the O.D. absorbance at 450nm in a microplate reader immediately. (If your microplate reader has a choice of correction wavelength, set it to 570nm or 630nm. Correct the read value to the OD450 value minus the OD570 or OD630 value. In this way, the OD value of non-chromogenic substances can be corrected and removed, thus obtaining more accurate results. If the microplate reader does not have a 570nm or 630nm wavelength, the original OD450 value can be used.)

Data Analysis

1. Calculatin of the Cutoff Value

Cutoff Value = **NCx**+0.1

NCx: Mean Absorbance of Negative Control(When the negative mean A value is less than 0.05, it is calculated as 0.05.When the negative mean A value is greater than or equal to 0.05, it is calculated according to the actual value.)

2. Determination of results

Sample with absorbance values ＜ Cutoff Value are NON-REACTIVE and are considered NEGATIVE for HCV.

Sample with absorbance values ≥Cutoff Value are considered POSITIVE for HCV.

3. Quality control

The blank well (only adding TMB and Stop solution) should not be greater than 0.08.

The positive control (PC) A value was greater than 0.3.

The negative mean A value was less than 0.08.

Assay Limitations

1. Any assay can't absolutely ensure antibodies or antigens with low concentration are not included in samples. Thus, negative result can't exclude the possibility that HCV has been exposed and infected.
2. This kit is only suitable for detecting individual serum or plasma sample. The detection result for other body fluids and samples may be inaccurate.