Human Anti HCV ELISA Kit

Cat #: orb564521 (manual)

For research use only. Not intended for diagnostic use.

Product Features

Catalog No.: orb564521

Size: 96 T

Reactivity: Human

Application: This immunoassay kit allows for the qualitative determination of HCV IgG in human serum or plasma.

Storage: 2-8℃

Principle: Indirect

Kit Components

Item	Specifications (96T)	Storage
Micro ELISA Plate (Dismountable)	12 × 8	2-8°C
HCV IgG Negative Control	0.2ml×1	2-8°C
HCV IgG Positive Control	0.2ml×1	2-8°C
Sample Dilution Buffer	20ml×1	2-8°C
HRP Anti-Human IgG Antibody	12ml×1	2-8°C(Avoid Direct Light)
Wash Buffer (25X)	30ml×1	2-8°C
TMB Substrate	10ml×1	2-8°C(Avoid Direct Light)
Stop solution	10ml×1	2-8°C
Plate Sealer	3 pieces	
Product Description	1 copy	

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Assay Principle

This kit was based on indirect enzyme-linked immune-sorbent assay technology. Recombined HCV antigen was pre-coated onto 96-well plates. The test samples were added to the wells, unbound conjugates were washed away with wash buffer. Then added HRP conjugated anti-human IgG Antibody, if there were any HCV IgG in the samples, it would form a complex. TMB substrates were used to visualize HRP enzymatic reaction. It was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.

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 manufacturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Materials Required but Not Supplied

1. Microplate reader (wavelength: 450nm)
2. 37°C 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

Manual Washing

Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of THREE washes.

Automated Washing

Aspirate all wells, wash plate three times with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

Sample Preparation

Isolate the test samples promptly after collection, and analyze them immediately (within 2 hours). Alternatively, aliquot and store at –20°C for long-term preservation. Avoid repeated 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°C.
- Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Analyze immediately or aliquot and store frozen at -20°C.

Note: Samples used within 5 days can be stored at 2-8°C; otherwise, they must be stored at -20°C or -80°C or liquid nitrogen to avoid loss of biological activity and contamination. Avoid multiple freeze-thaw cycles. Hemolytic samples are not suitable for this test.

Reagent Preparation

Wash Buffer Preparation:

If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

Dilute 30ml Concentrated Wash Buffer to 750ml Wash Buffer with deionized or distilled water. Put unused solution back at 2-8°C.

Assay Procedure

1. Bring all reagents to room temperature before use.
2. Take the required number of microporous strips and fix them to the bracket and number them sequentially
3. Add 100µL sample dilution buffer to each well.
4. Add 10µL sample, Negative Controls and Positive Controls or samples to be tested to the wells and gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37°C for 60 min.
5. Remove the cover, and wash plate 5 times with Wash buffer and each time let the wash buffer stay in the wells for 0.5-1min.
6. Add 100µL HRP- anti-human IgG Antibody to each well.
7. Seal the plate with a cover and incubate at 37°C for 30 min.
8. Remove the cover, and wash plate 5 times with Wash buffer and each time let the wash buffer stay in the wells for 0.5-1 min.
9. Add 90µl of TMB substrate into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in dark within 15 min. And the shades of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.

10. Add 50µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

11. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution (Use the blank well to set zero).

Calculation of Results

Cutoff Value (C.O.) = **NCx**×2.8

NCx: Mean Absorbance of Negative Control (When **NCx** ≤0.05, take 0.05 as default)

Note:

1. Sample with absorbance values S /C.O.< 1 are NON-REACTIVE and are considered NEGATIVE for HCV IgG. Sample with absorbance value S /C.O.≥ 1 are considered POSITIVE for HCV IgG.

2. Mean Absorbance of Negative Control >0.08 **OR** Mean Absorbance of Positive Control ≤ 0.5, it should be considered invalid and need to be retested.

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