Creative Diagnostics®

Version 30-05/24

User's Manual

IgM Antibody to Hepatitis C Virus, HCV-IgM ELISA Kit

REF **DEIA063**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

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

Principles of Testing

This kit was based on Capture ELISA. Anti-Human-IgM(μchain) 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- Conjugates, if there were any HCV IgM in the samples, it would form a Anti-Human-IgM(μchain)- HCV IgM- HRP- HCV-Ag 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.

Reagents And Materials Provided

1. Micro ELISA Plate(Dismountable): 12 × 8, 2-8°C/-20°C
2. HCV IgM Positive Control: 0.2 ml × 1, 2-8°C
3. HCV IgM Negative Control: 0.2 ml × 1, 2-8°C
4. Sample dilution buffer: 12 ml × 1, 2-8°C
5. HRP- HCV-Ag: 12 ml × 1, 2-8°C (Avoid Direct Light)
6. TMB substrate A: 6 ml × 1, 2-8°C (Avoid Direct Light)
7. TMB substrate B: 6 ml × 1, 2-8°C (Avoid Direct Light)
8. Stop solution: 6 ml × 1, 2-8°C
9. Wash buffer (20×): 50 ml × 1, 2-8°C
10. Plate Sealer: 2 pieces
11. Product Description: 1 copy

Materials Required But Not Supplied

1. Microplate reader (wavelength: 450 nm)
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

Storage

2-8°C

Specimen Collection And Preparation

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

1. Serum: Coagulate the serum at room temperature (about 1 hours). Centrifuge at approximately 1000 ×g for 15 min. Analyze the serum immediately or aliquot and store at -20°C.
2. Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15min at 2-8°C at 1500 ×g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 ×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: Dilute 50 mL of Concentrated Wash Buffer to 1000 mL of Wash Buffer with deionized or distilled water.

Assay Procedure

1. Label the sample wells, 3 Negative Controls, 2 Positive Controls and 1 blank wells.
2. Add 100 µL sample dilution buffer to each wells (except blank well)
3. Add 10 µL sample serum or plasma or Negative Controls and Positive Controls. Gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37±1 °C for 60 ±2 min.
4. Remove the cover, and wash plate 5 times with Wash buffer and try to dry it one last time.
5. Add 100 µL HRP-HCV-Ag to each well, except blank well
6. Seal the plate with a cover and incubate at 37±1°C for 30±1 min.
7. Remove the cover, and wash plate 5 times with Wash buffer and try to dry it one last time.
8. Add 50 µl of TMB substrate A and 50 µl of TMB substrate B into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37±1°C in dark within 30±1 min. And the shades of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.
9. Add 50 µl of Stop solution into each well and mix thoroughly. Results are measured within 10 minutes.
10. Read the O.D. absorbance at 450 nm (Recommended use 450 nm/600-650nm) in a microplate reader immediately after adding the stop solution. (Use the blank well to set zero)

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 350µl 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, then wash plate THREE times with 350 µl 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.

Calculation

1. Calculation of the Cutoff Value

Cutoff Value = NCx + 0.12 (Shortfall of 0.02 is calculated as 0.02)

2. Determination of results

(1) The negative control hole A value ≤ 0.08 ,The Positive control hole A value ≥ 0.50, Otherwise, the experiment is invalid.

(2) If the A value of 1 hole negative control is greater than 0.08, it should be abandoned, and if the A value of two holes or more negative control is greater than 0.08, the experiment should be repeated.

(3) Negative determination: the sample A value < critical value (CUTOFF) was negative for HCV antibody.

(4) Positive determination: the sample A value ≥ critical value (CUTOFF) were HCV antibody positive (note: the first test positive should be resampled double hole repeat test).

Limitations

1. After opening and before using, keep plate dry.
2. Before using the Kit, balance the reagents at room temperature at least 30 mins.
3. Storage TMB reagents avoid light.
4. Washing process is very important, not fully wash easily cause a false positive.
5. Don't let Micro plate dry at the assay, for dry plate will inactivate active components on plate.
6. Don't reuse tips and tubes to avoid cross contamination.
7. Avoid using the reagents from different batches together.