Human Hepatitis C Virus IgG (HCV-IgG) ELISA Kit

Catalog No.: abx054087

Size: 96T

Range: Qualitative

Sensitivity: Qualitative

Storage: Store at 2-8°C in the dark.

Application: For quantitative detection of HCV-IgG in serum and plasma.

Principle of the Assay

This kit is based on indirect enzyme-linked immuno-sorbent assay technology. The 96-well plate is pre-coated with the target antigen. Samples are added to the wells and incubated, then washed with wash buffer. Samples that contain HCV-IgG will bind to the pre-coated antigen to form an antigen-antibody complex. Next, HRP-conjugated detection antigen is added to the wells and incubated, then washed. Free components are washed away with wash buffer. TMB substrate is used to visualize HRP activity. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding stop solution. The intensity of the color yellow is proportional to the amount of HCV-IgG bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the presence of HCV-IgG can be calculated.

Kit components

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Positive control: 1 vial
3. Negative control: 1 vial
4. Detection reagent: 1 vial
5. Wash buffer (20X): 2 x 25 ml
6. TMB substrate A: 1 vial
7. TMB substrate B: 1 vial
8. Stop solution: 1 vial
9. Sample Diluent Buffer: 1 vial
10. Plate Sealer: 3
11. Hermetic Bag: 1

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. Distilled or deionized water
7. 1.5 ml tubes to prepare standard/sample dilutions
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

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Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.
- Samples should be clear and transparent. Please bring samples slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain NaN3 cannot be detected as it interferes with HRP.
- Always use non-pyrogenic, endotoxin-free tubes for blood collection.

2. Wash buffer

Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e. add 50 ml of concentrated wash buffer into 950 ml of distilled water).

B. Assay Procedure

Equilibrate the kit components and samples to room temperature for at least 30 minutes prior to use.

1. Determine the number of wells to be used. Any strips that are not being used should be kept dry and stored at 4°C.
2. Set up Negative Control, Positive Control, blank, and sample wells on the microplate.
3. Add 100 µl of Sample Dilution buffer to all wells.
4. Add 10 µl of Sample to each Sample well.
5. Add 10 µl of Positive Control and 10 µl of Negative Control to the Positive and Negative Control wells respectively.
6. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 60 minutes.
7. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with Wash buffer (300 µl) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
8. Add 100 µl of Detection Reagent to all wells. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 30 minutes.
9. Remove the cover and discard the liquid. Wash the plate 5 times as explained in step 5.
10. Add 50 µl of TMB substrate A and 50 µl of TMB substrate B to each well. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 30 minutes in the dark.
11. Remove the cover and add 50 µl of Stop solution to each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm within 10 minutes of adding the stop solution.

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C. Analysis

1. **Calculations:**
   Mean absorbance of the positive control should be > 0.50.
   Mean absorbance of the negative control should be < 0.08.
   Mean absorbance of the blank should be ≤ 0.08.
   CUT OFF value (negative control < 0.05) = 0.05 x 2.8
   CUT OFF value (negative control ≥ 0.05) = negative control x 2.8
2. **Interpretation of results:**
   If the positive control value is > 0.50, and negative control value is ≤ 0.08, the test is valid, otherwise, the test is invalid.
   Samples:
   If O.D. of samples < CUT OFF, the test samples are considered negative.
   If O.D. of samples ≥ CUT OFF, the test samples are considered positive.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimised. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.
- Please note that this kit is optimised for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

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