

ELISA Kit for Human Hepatitis C Virus (Core, E2, NS3, NS4, and NS5)

[NAME AND INTENDED USE]

***ELISA Kit for Antibody to Human Hepatitis C Virus *** is an *in vitro* enzyme immunoassay for the detection of Anti-HCV antibody IgG in human serum or plasma.

[PRINCIPLE]

This third generation kit uses indirect ELISA method to detect antibodies anti HCV in serum or plasma. The recombinant HCV antigens (Core, E2, NS3, NS4, and NS5) are coated on the multi-wells plate. HRP conjugated mouse anti-human IgG (gamma chain) serves as tracer. TMB is the substrate of HRP for color development. It is used for screen patients infected with HCV. The test has high sensitivity and specificity, reproducible and easy to operate.

[MATERIALS PROVIDED]

1. HCV Antigen Coated Microwell Plate	1 block (96wells)
2. Sample Diluent	1 bottle (12ml)
3. Enzyme Conjugant	1 bottle (12ml)
4. Negative Control Serum	1 vial (1ml)
5. Positive Control Serum	1 vial (1ml)
6. Concentrated Wash Buffer (1:20 dilution prior to use)	1 bottle (50ml)
7. Substrate A	1 bottle (6ml)
8. Substrate B	1 bottle (6ml)
9. Stop Solution	1 bottle (6ml)
10. Plastic Bag	1 bag
11. Seal Paper	3 pieces
12. Manual	1 each

[SAMPLE COLLECTION AND PRESERVATION]

Blood serum samples are routinely prepared from vein. Blood plasma sample are routinely prepared with routine amount of anticoagulant such as heparin or sodium citrate. Sample can be stored at 4°C if tested within five days. Sample can be stored at -20°C at least for 3 months. Avoid hemolysis and repetitive freeze and thaw of samples. Samples with cloud or precipitation should be centrifuged or filtered before test. Prevent serum from bacteria contamination during collection and storage.

[TEST PROCEDURE]

1. Bring *** ELISA Kit for Antibody to Human Hepatitis C Virus *** (all reagents), and samples to room temperature before use (approximately 30 minutes).
2. Dilute concentrated wash buffer 1:20 with ddH₂O
3. For each test, set one blank, two positive and three negative controls. Add 100 µl positive and negative control serum into positive and negative control wells respectively without sample diluent.
4. Add 100 µl of sample diluent into each test well, add 10 µl test serum into test wells, mix thoroughly,
5. Cover wells with seal paper, incubate for 30 minutes at 37°C.
6. Discard the liquid in all wells and fill the wells with wash solution (300µl per well). Lay aside for 15 seconds, discard the liquid in all wells and fill the wells with wash solution. Repeat 5 times and dry wells after last wash.
7. Add enzyme conjugant 100 µl into the wells except the blank well.
8. Cover wells with seal paper, incubate for 20 minutes at 37°C.
9. Wash plate 5 times as described in Step 6.
10. Add substrate A and B one drop or 50 µl respectively to each well, mix gently, protected from light and lay aside for 15 minutes at 37°C.
11. Add one drop of stop solution (50 µl) into each well to stop the

reaction, including blank well.

12. Measure the absorbance at 450 nm against the blank, or measure the absorbance at 450 nm/630 nm.

[INTERPRETATION OF RESULTS]

Colorimetric Method

Cut Off Value calculation:

COV = 0.1 + the average OD of negative controls, (if the average OD of negative controls is below or equal to 0.05, calculate it as 0.05),

Positive OD₄₅₀ of sample ≥ COV

Negative OD₄₅₀ of sample < COV

[PRECAUTIONS]

1. The samples should be fresh, avoid hemolysis, bacteria growing, and repetitive freeze and thaw.
2. Do not interchange reagents between kit lots. The seal paper can't be used repeatedly.
3. Mix reagents well before use. If crystal form in certain reagents, such as wash buffer, it can be used without problems after warm up and mix well.
4. Follow instruction exactly during assay, especially in temperature and time for reactions. All pipetting devices should be used with care and calibrated regularly following the manufacturer's instructions.
5. Put the remained reagents to the sealed pouch, and return to 2-8°C in time.
6. To prevent cross-contamination, wear gloves and working suits throughout the procedure, and execute the disinfection and isolation regulations strictly. Dispose of all samples and materials used to perform the test. The 5.0g/L liquid sodium hypochlorite solution or 121°C high pressure steam may be used to disinfect samples and materials before disposal

[STORAGE AND STABILITY]

Store the kit at 2-8°C. The kit is stable within the expiration date printed on kit boxes. **Do not freeze** or use the kit beyond the expiration date.

[EXPIRATION]

The shelf life is 12 months. Do not use the kit beyond its expiration date.

This Kit is for Research Use Only