

## Human Anti-HCV-IgM ELISA Kit

### Order details

**CatNo:** AHY-26

**Size:** 96T/48T

### Product description

This immunoassay kit allows for the qualitative determination of HCV IgM in human serum or plasma. Is based on Capture ELISA. Anti-Human-IgM( $\mu$  chain) 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( $\mu$  chain)-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.

### Product description

Microplate	8x12/12x8-well strips per plate
Negative Control	0.2ml, 1 vial
Positive Control	0.2ml, 1 vial
HRP-Conjugate	12ml, 1 vial

Sample Diluent	12ml,1 vial
Substrate Solution A	6ml, 1 vial
Substrate Solution B	6ml, 1 vial
Stop Solution	6ml, 1 vial
Wash Buffer (20×)	50ml, 1 vial
Microtiterplate sealers	2 sheets
Plastic Sealable Bag	1 unit

Storage method: it can be stored at 2-8 °C for more than 6 months. Do not freeze it.

## Steps

1. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve.
2. Set the strips needed in strip-holder and number sufficient number of wells including three Negative control (e.g. B1, C1, D1), two Positive control (e.g. E1, F1) and one Blank (e.g. A1, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.
3. Add Positive and Negative controls into their respective wells, add Sample Diluent into other reaction wells, then add sample, mix well.

Note: Use a separate disposable pipette tip for each specimen, Negative and Positive Control as to avoid cross-contamination.

4. Cover the plate with the plate cover and incubate at 37°C.
5. Add HRP-HCV-Ag to each well, except blank well, seal the plate with a cover and incubate at 37±1°C. Remove the cover, and wash plate 5 times with Wash buffer and try to dry it one last time.
6. Add TMB substrate A and 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. And the shades

of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.

7. Add Stop solution into each well and mix thoroughly. Results are measured within 10 minutes.

8. Calibrate the plate reader with the Blank well and read the absorbance at 450 nm. If a dual filter instrument is used, set the reference wavelength at 630 nm. Calculate the Cutoff value and evaluate the results. (Note: read the absorbance within 5 minutes after stopping the reaction.)

## Note

1. The kit should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay. It is highly recommended that the solutions be used as soon as possible after rehydration.
2. When mixing or reconstituting protein solutions, always avoid foaming.
3. Do not mix or substitute reagents with those from other lots or sources.
4. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions.
5. Crystals could appear in the 20X wash solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
6. Keep TMB Substrate protected from light.
7. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.