HCV core antigen ELISA Kit

Enzyme Immunoassay for the quantification of HCV core antigen in purified virus or unpurified viral supernatant.

Catalog number: ARG81362

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INTRODUCTION

Hepatitis C virus (HCV) is a small (55–65 nm in size), enveloped, positive-sense single-stranded RNA virus of the family Flaviviridae. Hepatitis C virus is the cause of hepatitis C and some cancers such as liver cancer (hepatocellular carcinoma, abbreviated HCC) and lymphomas in humans.

The hepatitis C virus particle consists of a lipid membrane envelope that is 55 to 65 nm in diameter. Two viral envelope glycoproteins, E1 and E2, are embedded in the lipid envelope. They take part in viral attachment and entry into the cell. Within the envelope is an icosahedral core that is 33 to 40 nm in diameter. Inside the core is the RNA material of the virus.

Chronic hepatitis C virus (HCV) infection diagnosis including HCV antibody and HCV RNA (HCV Nucleic Acid Testing) detection. Currently, several studies have shown testing for HCV core antigen is a potential alternative method to HCV Nucleic Acid Testing, and it might enhance the diagnostic yield of persons with acute HCV when compared with HCV antibody testing alone. [Modified from Wikipedia and hepatitisc.uw.edu: Hepatitis C online]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for HCV core antigen (HCVcAg) has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any HCV core antigen present is bound by the immobilized antibody. After washing away any unbound substances, a FITC-conjugated antibody specific for HCV core antigen is added to each well and incubate. Following a washing to

remove unbound substances, a HRP-conjugated mouse anti-FITC antibody is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of HCV core antigen bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of HCV core antigen in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
antibody-coated microplate	12 X 8 strips	4°C
Standard (10 µg/ml)	100 µl	-20°C
10X Wash Buffer	100 ml	4°C
1000X FITC-conjugated-HCV core antigen Antibody concentrate	20 µl	4°C
1000X HRP-conjugated-FITC Antibody concentrate	20 µl	4°C
Triton X-100 Solution (5%)	15 ml (Ready-to-use)	4°C
Assay Diluent	50 ml (Ready-to-use)	4°C
TMB substrate	12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: 620 nm as optional reference wave length)
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water.
- Microplate shaker.
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Standard at -20°C and other kit components at 4°C at all times.
- Upon receipt, the Standard should be aliquoted and stored at ≤ -20°C to avoid repeated freeze-thaw cycles.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards and samples be assayed in duplicates.

- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

1. (Optional) Dilute HCV sample in culture medium. Include culture medium as a negative control.

2. Transfer 225 µl of each sample to a microcentrifuge tube containing 25 µl of Triton X-100 Solution, Vortex to mix well.

3. Incubate the tube for 30 minutes at 37°C.

Note: For samples that contain anti- HCV core antigen (HCVcAg) antibody, to release HCV core antigen from the virion and antibody. The sample should be incubated at 56°C for 30 min before assay to inactivate anti-HCVcAg antibodies.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer, mix well. Storage at 2-8°C.
- 1X FITC-conjugated-HCV core antigen Antibody working solution: Dilute the antibody immediately before use; dilute the 1000X FITC-conjugated-HCV core antigen Antibody concentrate into Assay Diluent to yield 1X FITC-conjugated HCV core antigen antibody working solution. (E.g.: 10 µl of the FITC-conjugated-HCV core antigen Antibody concentrate (1000X) + 9.990ml of Assay Diluent) Do not store diluted solutions.
- 1X HRP-conjugated-FITC Antibody working solution: Dilute the antibody immediately before use; dilute the 1000X HRP-conjugated-FITC Antibody concentrate into Assay Diluent to yield 1X HRP-conjugated-FITC Antibody

working solution. (E.g.: 10 µl of the HRP-Streptavidin concentrate (1000X) + 9.990 ml of Assay Diluent.) Do not store diluted solutions.

- Human HCV core antigen standard:

1. Prepare a series dilution of Human HCV core antigen standards with Assay Diluent. The Assay Diluent serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with Assay Diluent as according to the suggested concentration table below:

Standard No	Human HCV core antigen (ng/ml)	Assay Diluent (µl)	Standards (µl)
S1	100	990	10 (10 µg/ml stock)
S2	50	500	500 µl (S1)
S3	25	500	500 µl (S2)
S4	12.5	500	500 µl (S3)
S5	6.25	500	500 µl (S4)
S6	3.125	500	500 µl (S5)
S7	1.5625	500	500 µl (S6)
S0	0	500	0

2. Transfer 225µL of each dilution to a microcentrifuge tube containing 25 µl of Triton X-100 Solution. Perform the assay as described in Assay Instructions.

ASSAY PROCEDURE

Warm Substrate Solution to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. Standards and samples should be assayed in duplicates.
2. Add **100 µl of inactivated sample or HCVcAg standard** into the appropriate wells in the antibody coated plate. Cover the plate and incubate for **2 hour at 37°C**.
3. Aspirate each well and wash, repeating the process 4 times for a total **5 washes**. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting
4. Add **100 µl** of the 1:1000 **diluted 1X FITC-conjugated-HCV core antigen antibody working solution** to each well, cover the plate and incubate for **1 hour at RT** on a microplate shaker.
5. Aspirate each well and **wash** as step 3.
6. Add **100 µl** of the 1:1000 **diluted 1X HRP-conjugated-FITC antibody working solution** to all wells, cover the plate and incubate for **1 hour at RT** on a microplate shaker.
7. **Warm TMB substrate solution to RT** before next wash step. Aspirate each well and wash as step 3. Proceed immediately to the next step.
8. Add **100 µl** of **TMB substrate solution** into each well. Incubate for **5-20 mins at RT** on microplate shaker. Avoid exposure to light.

Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9. Add 100 µl of Stop Solution to each well and shake lightly to ensure homogeneous mixing.
10. Read the OD with a microplate reader at 450nm immediately (optional: read at 620 nm as reference wave length).

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

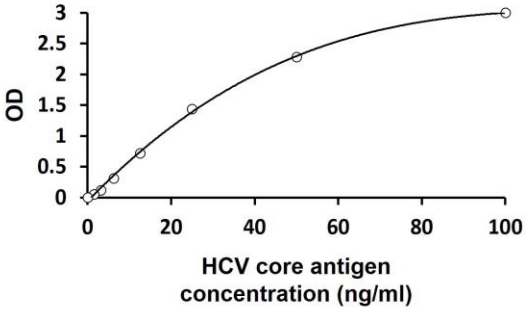
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

1 ng/ml

Assay Range

1.56- 100 ng/ml