Product Manual

QuickTiter™ Hepatitis C Core Antigen (HCVcAg) ELISA Kit

Catalog Numbers

VPK-151	96 wells

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Hepatitis C is an infection of the liver caused by the hepatitis C virus. HCV is transmitted by exposure to infectious blood; forms of transmission include unprotected sexual activity, blood transfusion, mother-to-infant transmission, or occupational exposure to blood. The acute illness causes liver inflammation, liver fibrosis, vomiting and jaundice, while chronic HCV infection often leads to liver cirrhosis and failure.

An estimated 270-300 million people worldwide have been infected with hepatitis C. At least 75% of people infected will develop chronic hepatitis C and have it the rest of their lives.

Diagnosis of chronic hepatitis C virus (HCV) infection has long been based on HCV serology and detection of HCV antibodies. With the development of therapies for chronic HCV infection, including interferon and ribavirin, quantitative detection of HCV has been used increasingly as the most important marker for monitoring HCV titer, disease progression, and assessing antiviral treatment. Several assays for the quantitative measurement of HCV DNA have been developed, such as PCR-based nucleic acid amplification assays. However, these methods tend to be cumbersome and expensive.
Cell Biolabs' QuickTiter™ HCV Core Antigen ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the HCV core protein. The kit has detection sensitivity limit of 1 ng /mL HCVcAg. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and HCV samples.

Assay Principle

An anti-HCVcAg monoclonal coating antibody is adsorbed onto a microtiter plate. HCV core antigen present in the sample or standard binds to the antibodies adsorbed on the plate; a FITC-conjugated mouse anti-HCVcAg antibody is added and binds to the antigen captured by the first antibody.

Following incubation and wash steps, a HRP-conjugated mouse anti-FITC antibody is added and binds to the FITC conjugated anti-HCVcAg. Unbound HRP-conjugated mouse anti-FITC antibody is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

A colored product is formed in proportion to the amount of HCV core antigen present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from recombinant HCV core antigen and sample concentration is then determined.

Related Products

1. VPK-150: QuickTiter™ HBV Core Antigen ELISA Kit
2. VPK-108-H: QuickTiter™ Lentivirus Quantitation Kit (HIV-1 p24 ELISA)
3. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
4. VPK-112: QuickTiter™ Lentivirus Quantitation Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-HCVcAg Antibody Coated Plate (Part No. 315101): One strip well 96-well plate.
2. FITC-Conjugated Anti-HCVcAg Monoclonal Antibody (Part No. 315102): One 20 µL vial.
3. HRP-Conjugated Anti-FITC Monoclonal Antibody (Part No. 310811): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. Triton X-100 Solution (Part No. 310805): One 15 mL bottle containing 5% Triton X-100 in TBS.
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Recombinant HCVcAg Standard (Part No. 315103): One 100 µL vial of 10 µg/mL recombinant HCV Core Antigen in 8 M Urea containing BSA.

Materials Not Supplied

1. HCV Sample: purified virus or unpurified viral supernatant
2. Cell Culture Centrifuge
3. 0.45 µm filter
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir
7. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, aliquot and store recombinant HCVcAg Standard at -20ºC and avoid freeze/thaw. Store all other components at 4ºC.

Safety Considerations

Remember that your samples contain infectious viruses before inactivation; you must follow the recommended NIH guidelines for all materials containing infectious organisms.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.

- FITC-Conjugated Anti-HCVcAg Monoclonal Antibody and HRP-Conjugated Anti-FITC Monoclonal Antibody: Immediately before use dilute the FITC-conjugated antibody 1:1000 and HRP-conjugated antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of Recombinant HCVcAg Standard in the concentration range of 100 ng/mL to 1 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	HCVcAg Standard (µL)	Assay Diluent (µL)	HCVcAg (ng/mL)
1	10	990	100
2	500 of Tube #1	500	50
3	500 of Tube #2	500	25
4	500 of Tube #3	500	12.5
5	500 of Tube #4	500	6.25
6	500 of Tube #5	500	3.125
7	500 of Tube #6	500	1.5625
8	0	500	0

Table 1. Preparation of HCVcAg Standard

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.

HCV Sample Dilution and Inactivation

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 well.
3. Incubate 30 minutes at 37ºC.

   Note: For samples that contain antibodies, release HCVcAg from the virion by incubating samples at 56ºC for 30 min.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Each HCV sample, HCVcAg standard, blank, and control medium should be assayed in duplicate.
3. Add 100 µL of inactivated sample or HCVcAg standard to Anti-HCVcAg Antibody Coated Plate.
4. Cover with a Plate Cover and incubate at 37ºC for 2 hours.

5. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
6. Add 100 µL of the diluted FITC-Conjugated Anti-HCVcAg Monoclonal Antibody to each well.
7. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
8. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 5 above.
9. Add 100 µL of the diluted HRP-Conjugated Anti-FITC Monoclonal Antibody to all wells.
10. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
11. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 5 above. Proceed immediately to the next step.
12. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
13. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
14. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical HCVcAg ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

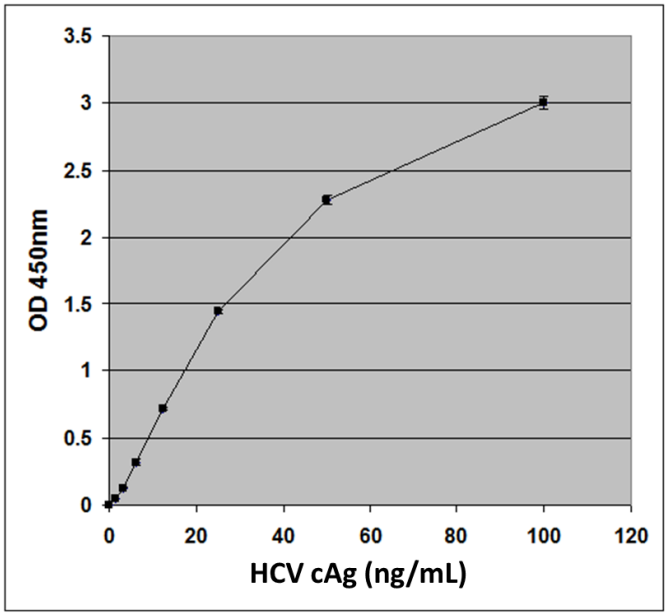

Figure 1: HCV Core Antigen ELISA Standard Curve

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Recent Product Citations

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