



(For scientific research use only, not for clinical diagnosis!)

**Human Hepatitis C Virus Core Antigen (HCV cAg) Qualitative
Detection Kit (ELISA) Instructions for Use Specifications: 48T/96T**

Please read the instructions carefully before use. If you have any questions, please contact us through the following methods: Official hotline: 400-999-8863 Technical phone: 18358180525 Email: UpingBio@163.com Company website: www.upingbio.com For the specific shelf life, please see the outer packaging label of the kit. Please use the kit within the shelf life.

When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.

Kit performance Physical properties: Each liquid component is clear and transparent, with no precipitation or floc. Microplate aluminum foil bags should be vacuum packed without damage or leakage.

Negative/positive control: The OD value of the negative control is not higher than 0.2, and the OD value of the positive control is not lower than 0.8.

Precision: intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%.

Recovery rate: The recovery rate is between 85%-115%.

Sensitivity: This kit recognizes natural human hepatitis C virus core antigen (HCV cAg) and has no overlap with structural analogs.

Stability: Stored at 2°C-8°C, validity period is 6 months.

Purpose: Used to detect the negative and positive levels of human hepatitis C virus core antigen (HCV cAg) in serum, plasma, cell culture supernatant and other samples.

Experimental principle This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). In the microwell enzyme plate pre-coated with anti-human hepatitis C virus core antigen (HCV cAg) antibody (solid-phase antibody), add human hepatitis C virus core antigen (HCV cAg) calibrator and the sample to be tested, and then Add anti-human hepatitis C virus core antigen (HCV cAg) antibody (enzyme-labeled antibody), and after incubation and sufficient washing, unbound components are removed, and a solid-phase antibody-antigen-enzyme label is formed on the solid surface of the microplate. Antibody sandwich complexes. Add substrates A and B. Under the catalysis of HRP, the substrate produces a blue product. Under the action of the stop solution, it is finally converted into yellow. The absorbance (OD value) is measured at a

wavelength of 450nm with a microplate reader. Through the absorbance (OD value) Determine whether the sample is negative or positive.

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Kit components and storage: Store unopened kits at 2-8

degrees Celsius. Do not use expired kits.

Components	quantity	Main ingredients	Store after opening
negative control	0.3ml/tube	--	2-8°C14 days
positive control	0.3ml/tube	--	2-8°C14 days
coated microplate	96T/48T	Pre-coated solid phase antibodies	2-8°C14 days
HRP labeled antibodies	10mL	HRP-labeled detection antibodies	2-8°C180 days
sample diluent	6mL	--	2-8°C180 days
Substrate solution A	6mL	0.01% hydrogen peroxidase	2-8°C180 days
Substrate solution B	6mL	0.1%TMB	2-8°C180 days
stop solution	6mL	acidic solution	2-8°C180 days
20×concentrated washing liquid	25mL	0.05%Tween20	2-8°C180 days
manual	1 serving	--	--
Ziplock bag	1	--	--
self-adhesive	2 tablets	--	--

The negative and positive controls have passed the test, and the results show that HBs antigen is negative, and HIV1, HIV2 and HCV antibodies are negative. Since there is no test method that can completely guarantee the absence of these substances, this product must be treated as potentially infectious, and the handling process should be Follow common safety measures.

Note: 1: Before use, please check whether the label and quantity of

the reagents in the kit are consistent with the table.

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2: If the components of the kit need to be used again, please ensure that they have not been contaminated since the last use. 3: If the enzyme plate is not used up in a single time, remember to seal it and store it at 2-8°C.

Self-prepared test equipment required for the test

(not provided, but can be purchased with

assistance) 1. Standard specification microplate

reader.

2. Automatic plate washing machine.

3. Oscillator.

4. A series of adjustable pipettes and tips. When testing a large number of samples at one time, it is best to use a multi-channel pipette.

Restrictions on the kit: 1. For scientific

research use only and not for clinical

diagnosis.

2. Use within the validity period marked on the kit. Expired products must not be used.

3. Do not mix with kits or components from other manufacturers.

4. Use the sample diluent provided with the kit.

5. If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately and then measure again. 6. Human anti-mouse and other

heterophilic antibodies present in the sample to be tested will interfere with the test results.

Please eliminate this factor before testing.

7. The test results obtained by other methods are not directly comparable to the test results of this kit.

Notes: 1) This kit is for in vitro research only and not for clinical diagnosis.

2) Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.

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- 3) Carry out incubation strictly according to the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Store reagents refrigerated immediately after use.
- 4) Improper plate washing can lead to inaccurate results. Make sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.
- 5) Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.
- 6) The substrate chromogenic solution should be colorless or very light in color.
- 7) Avoid cross-contamination of reagents and specimens to avoid erroneous results.
- 8) Avoid direct exposure to strong light during storage and incubation.
- 9) Equilibrate to room temperature before opening the sealed bag to prevent water droplets from condensing on the cold slats.
- 10) Any reaction reagents must not come into contact with bleaching solvents or strong gases emitted by bleaching solvents. Any bleaching ingredients will destroy the biological activity of the reagents in the kit.
- 11) The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of $450\pm 10\text{nm}$, and the optical density range is between 0-3.5. It is recommended to preheat 15 minutes in advance before use.
- 12) Do not mix or replace the reagents in this kit with reagents from other batch numbers or other sources.
- 13) The EP tubes and tips used in the test are single-use and are strictly prohibited from mixing.
- 14) Do not use expired reagents.

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Sample preparation and storage

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide must not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

Cell culture supernatant - centrifuge to remove precipitate, analyze immediately or aliquot and store frozen at -20°C.

Serum - Collect blood in a clean test tube, coagulate at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect serum. Analyze immediately or aliquot and store frozen at -20°C.

Plasma—anticoagulate with heparin, citrate, or EDTA, and centrifuge at 2000×g for 20 minutes at 2-8°C within 30 minutes of blood draw. To eliminate the influence of platelets, it is recommended to further centrifuge at 10,000 × g for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.

Cell lysis buffer - For adherent cells, remove the culture medium and wash with PBS, normal saline or serum-free culture medium. Add an appropriate amount of lysis solution and pipet several times with a gun to fully contact the lysate and cells. Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, blow the cells with a gun, and flick them with your fingers to fully lyse the cells. After full lysis, centrifuge at 10000-14000×g for 3-5 minutes and take the supernatant. Analyze immediately or aliquot and store frozen at -20°C.

Urine - Collect in sterile tubes and centrifuge at 2000×g for 20 minutes. Carefully collect the supernatant. If a precipitate forms, centrifuge again.

Reagent preparation 1. Before use, all components must be rewarmed for at least 120 minutes to ensure sufficient rewarming to room temperature.

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating in a water bath will completely dissolve the crystals. Dilute concentrated washing liquid and distilled water at 1:20, that is, add 1 part of concentrated washing liquid to 19 parts of distilled water.

3. Substrate: Substrate solutions A and B, mix thoroughly at a volume of 1:1 before use, and use within 15 minutes after mixing.

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Operating procedures: Return all reagents and components to room

temperature first. For negative controls, positive controls and samples, it is

recommended to make duplicate holes.

1. Prepare the working solutions of various components of the kit according to the method described in the previous instructions.

2. Take out the required slats from the aluminum foil bag, seal the remaining slats in a ziplock bag and return it to the refrigerator.

Set negative control wells, positive control wells, blank wells and sample wells. Add 50 μL of standards of different concentrations to each of the negative and positive control wells, do not add it to the blank well, and add 50 μL of the sample to be tested to the sample well.

3. In addition to the blank wells, add 100 μL of horseradish peroxidase (HRP)-labeled detection antibody to the negative control wells, positive control wells and sample wells.

4. Cover the reaction plate with sealing film and incubate it in a 37°C water bath or incubator for 60 minutes.

5. Uncover the sealing film, discard the liquid, pat dry on absorbent paper, fill each well with washing solution, let stand for 20 seconds, shake off the washing solution, pat dry on absorbent paper, repeat this 5 times. If you use an automatic plate washer, please wash the plate according to the plate washer operating procedure. Adding a soaking program for 30 seconds can improve the detection accuracy.

After washing the plate and before adding substrate, pat the reaction plate dry on clean, lint-free paper.

6. Mix substrates A and B thoroughly at a volume of 1:1, and add 100 μL of substrate mixture to all wells. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator for 15 minutes.

7. Add 50 μL of stop solution to all wells, and read the absorbance (OD value) of each well at a wavelength of 450 nm on a microplate reader.

[Interpretation of test results] After the test is completed, the OD of the negative control + 0.25 is used as the threshold. If the OD value of the sample is greater than the threshold, it is considered positive; if the OD value of the sample is lower than the threshold, it is considered negative.

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[Problem Analysis] If the experimental results are not good, please take pictures of the color development results in time, save the experimental data, keep the used strips and unused reagents, and then contact our company's technical support to solve the problem for you. At the same time, you can also refer to the following information: [Questions and Answers]

Problem Description	Possible Causes	Corresponding countermeasures Corresponding countermeasures
Positive control color difference	Incorrect liquid aspiration or	Check pipettes and tips
	Equilibration time is too short	Ensure sufficient balancing time
	Incomplete washing	Ensure the washing time and number of washings and the amount of liquid added to each hole
Very weak or colorless	Incubation time too short	Ensure adequate incubation time
	The experimental temperature is incorrect	Use recommended experimental temperatures
	Insufficient reagent volume or missing addition	Check the liquid aspirating and adding process to ensure that all reagents are added in order and in
	Incorrect dilution	
Enzyme label inactivation or substrate failure	Mix enzyme conjugate and substrate and check by rapid color development	
Reading value is low	Microplate reader settings are incorrect	Check the wavelength and filter
		Turn on the microplate reader and preheat it in advance
Large coefficient of variation	Adding fluid incorrectly	Check the filling situation
High background value	The working concentration of the	Use the recommended dilution
	Incomplete washing of enzyme plate	Ensure that each step of cleaning is complete; if using an automatic plate washer, please check whether all outlets are blocked;
	The lotion is contaminated	Prepare fresh lotion
Low sensitivity	Improper storage of ELISA kits	Store relevant reagents according to
	Not terminated before reading	Stop solution should be added to

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