



User's Manual

Human Anti-Hepatitis C Virus Antibody, Anti-HCV ELISA Kit



DEIA015s



96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Anti-HCV ELISA is an enzyme-linked immunosorbent assay (ELISA) for qualitative detection of antibodies to hepatitis C virus in human serum or plasma. It is intended for screening blood donors and diagnosing patients related to infection with hepatitis C virus.

General Description

Hepatitis C virus (HCV) is an envelope, single stranded positive sense RNA (9.5 kb) virus belonging to the family of Flaviviridae. Six major genotypes and series of subtypes of HCV have been identified. Isolated in 1989, HCV is now recognized as the major cause for transfusion associated non-A, non-B hepatitis. The disease is characterized with acute and chronic form although more than 50% of the infected individuals develop severe, life threatening chronic hepatitis with liver cirrhosis and hepatocellular carcinomas. Since the introduction in 1990 of anti-HCV screening of blood donations, the incidence of this infection in transfusion recipients has been significantly reduced. The first generation of HCV ELISAs showed limited sensitivity and specificity and was produced using recombinant proteins complementary to the NS4 (c100-3) region of the HCV genome as antigens. Second generation tests, which included recombinant / synthetic antigens from the Core (c22) and nonstructural regions NS3 (c33c, c100-3) and NS4 (c100-3, c200) resulted in a remarked improvement in sensitivity and specificity. Clinical studies show that significant amount of HCV infected individuals develop antibodies to NS5 non-structural protein of the virus. For this, the third generation tests include antigens from the NS5 region of the viral genome in addition to NS3 (c200), NS4 (c200) and the Core (c22). Third generation tests have improved sensitivity and shorten the time between infection with HCV and the appearance of detectable antibodies (window period) to 60 days.

Reagents And Materials Provided

1. MICROWELL PLATE: 8×12/12×8-well per plate. Blank microwell strips fixed on white strip holder.

The plate is sealed in aluminum pouch with desiccant. Each well contains recombinant HCV antigens. The microwell strips can be broken to be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2-8°C. Once open, stable for one month at 2-8°C.

2. NEGATIVE CONTROL: 1×0.2ml. Blue-colored liquid filled in a vial with green screw cap. Protein-stabilized buffer tested nonreactive for HCV antibodies. Ready to use as supplied. Once open, stable for one month at 2-8°C.

3. POSITIVE CONTROL: 1×0.2ml. Red-colored liquid filled in a vial with red screw cap. Anti-HCV antibodies diluted in protein-stabilized buffer. Ready to use as supplied. Once open, stable for one month at 2-8°C.

4. HRP-CONJUGATE: 1×13ml. Red-colored liquid in a white vial with red screw cap. Horseradish peroxidase-conjugated rabbit anti-human IgG antibodies. Ready to use as supplied. Once open, stable for one month at 2-8°C.

5. SPECIMEN DILUENT: 1×13ml. Green-colored in a vial with blue screw cap. Serum base, casein, and sucrose solution.

6. WASH BUFFER: 1x50ml. Colorless liquid filled in a clear bottle with white screw cap. PH 7.4, 20 x PBS

7. CHROMOGEN SOLUTION A: 1x8ml. Colorless liquid filled in a white vial with green screw cap. Urea peroxide solution. Ready to use as supplied. Once open, stable for one month at 2-8°C.

8. CHROMOGEN SOLUTION B: 1x8ml. Colorless liquid filled in a black vial with black screw cap. TMB solution (Tetramethyl benzidine dissolved in citric acid). Ready to use as supplied. Once open, stable for one month at 2-8°C.

9. STOP SOLUTION: 1x8ml. Colorless liquid in a white vial with yellow screw cap. Diluted sulfuric acid solution (0.5M H₂SO₄). Ready to use as supplied. Once open, stable for one month at 2-8°C.

10. PLASTIC SEALABLE BAG: 1 unit. For enclosing the strips not in use.

11. PACKAGE INSERT: 1 copy.

12. CARDBOARD PLATE COVER: 2 sheets. To cover the plates during incubation and prevent evaporation or contamination of the wells.

Materials Required But Not Supplied

1. Freshly distilled or deionized water
2. Disposable gloves and timer
3. Appropriate waste containers for potentially contaminated materials
4. Dispensing system and/or pipette
5. Disposable pipette tips
6. Absorbent tissue or clean towel
7. Dry incubator or water bath, 37±0.5°C
8. Plate reader, single wavelength 450nm or dual wavelength 450/630nm
9. Microwell aspiration/wash system.

Storage

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, do not freeze. To assure maximum performance of this Anti-HCV ELISA, during storage, protect the reagents from contamination with microorganism or chemicals.

Specimen Collection And Preparation

1. Specimen Collection: No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely-the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the specimen should be removed by centrifugation at 3000 rpm (round per minutes) for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolytic specimens should not be used as they can give false results in the assay. Do not heat



inactivate specimens. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.

3. This Anti-HCV ELISA is intended **ONLY** for testing of individual serum or plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
4. **Transportation and Storage:** Store specimens at 2-8°C. Specimens not required for assaying within 3 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

Reagent Preparation

Note:

Allow the reagents to reach room temperature (18-30°C). Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash buffer (20×) as indicated in the instructions for washing. Use distilled or deionized water and only clean vessels to dilute the buffer. All other reagents are **READY TO USE AS SUPPLIED**

WASH BUFFER: The concentrate must be diluted 1 to 20 with distilled/ deionized water before use. Once diluted, stable for one week at room temperature, or for two weeks when stored at 2-8°C.

Assay Procedure

INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential in order to obtain correct and precise analytical data.
2. It is therefore, recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles of 350-400µl/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with specimen or HRP-conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to carry out 5 washing cycles, dispensing 350-400µl/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before they are wasted in an appropriate way.
7. The concentrated Wash buffer should be diluted 1:20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

PROCEDURE:

Step1 Preparation: Mark three wells as Negative control (e.g. B1, C1, D1), two wells as 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.

Step2 Adding Diluent: Add 100µl of Specimen Diluent into each well except the Blank.

Step3 Adding Sample: Add 10µl of Positive control, Negative control, and Specimen into their respective wells except the Blank. **Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination. Mix by tapping the plate gently.**

Step4 Incubating: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.

Step5 Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remainders.

Step6 Adding HRP-Conjugate: Add 100µl HRP-Conjugate into each well except the Blank.

Step7 Incubating: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.

Step8 Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Washing buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.

Step9 Coloring: Add 50µl of Chromogen A and 50µl Chromogen B solutions into each well including the Blank. Incubate the plate at 37°C for 15 minutes avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and anti-HCV positive sample wells.

Step10 Stopping Reaction: Using a multichannel pipette or manually, add 50µl Stop solution into each well and mix gently. Intensive yellow color develops in Positive control and anti-HCV positive sample wells.

Step11 Measuring the Absorbance: Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the Cut-off value and evaluate the results. **(Note: read the absorbance within 10 minutes after stopping the reaction).**

Quality Control

The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

1. The A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450 nm.
2. The A values of the Positive control must be ≥ 0.800 at 450/630nm or at 450nm after blanking.
3. The A values of the Negative control must be < 0.100 at 450/630nm or at 450nm after blanking.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded and the mean value calculated again using the remaining two values. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Calculation

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) value to the Cutoff value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well A value from the print

report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and controls.

Calculation of the Cut-off value (C.O.) = $N_c + 0.12$ (N_c = the mean absorbance value for three negative controls). **Important: If the mean A value of the negative controls is lower than 0.02, take it as 0.02.**

Interpretation Of Results

Negative Results (A / C.O. < 1): Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no anti-HCV antibodies have been detected with this Anti-HCV ELISA, therefore the patient is probably not infected with HCV and the blood unit do not contain antibodies to HCV and could be transfused in case that other infectious diseases markers are also absent.

Positive Results (A / C.O. \geq 1): Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that anti-HCV antibodies have probably been detected using this Anti-HCV ELISA. All initially reactive specimens should be retested in duplicates using this Anti-HCV ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for antibodies to HCV with this Anti-HCV ELISA.

Borderline (A / C.O. = 0.9-1.1): Specimens with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results.

Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system (e.g. RIBA, PCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

1. If, after retesting of the initially reactive samples, both wells are negative results (A/C.O.<0.9), these samples should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step.
2. If after retesting in duplicates, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for antibodies to HCV and therefore the patient is probably infected with HCV and the blood unit must be discarded.
3. After retesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.

Performance Characteristics

1. Performance on BBI low and mixed titers anti-HCV panels.

BBI PHV105 anti-HCV low titer performance panel

	BBI Ref. Data			Anti-HCV ELISA	
No.	Western Blot	EIA Result, A/C.O.			
	RIBA 3.0	EIA 2.0	EIA 3.0	Lot 1	Lot 2
1	POS	2.2	>4.7	6.6	4.5
2	POS	>4.9	4.3	6.3	14.9
3	POS	1.3	4.5	6.7	5.4
4	POS	2.7	>4.7	4.2	4.7
5	POS	2.1	4.4	6.6	5.5
6	POS	2.1	3.3	4.8	6.0
7	IND	2.7	1.7	4.0	2.8
8	IND	3.8	2.9	3.6	3.3
9	IND	1.1	1.7	2.5	2.5
10	NEG	0.1	0.0	0.0	0.0
11	IND	2.3	>4.7	3.6	3.0
12	POS	2.5	4.4	2.4	2.0
13	IND	2.2	3.5	1.4	1.6
14	IND	2.7	3.7	5.6	4.2
15	POS	2.8	1.8	2.3	2.8

BBI PHV205 anti-HCV mixed titer performance panel

	BBI Ref. Data			Anti-HCV ELISA	
No.	Western Blot	EIA Result, A/C.O.			
	RIBA 3.0	EIA 3.0	EIA 3.0	Lot 1	Lot 2
1	POS	6.8	>4.9	7.2	6.4
2	NEG	0.1	0.1	0.0	0.0
3	POS	6.8	>4.9	7.8	11.3
4	POS	6.6	>4.9	8.0	10.9
5	IND	3.3	2.1	7.2	3.6
6	IND	>4.9	1.5	5.5	4.2
7	POS	1.5	2.4	5.4	4.2
8	IND	4.0	2.3	5.5	4.1
9	POS	>4.9	2.2	3.3	2.1
10	POS	3.9	4.5	7.6	6.8
11	POS	>4.9	>4.9	7.0	10.4
12	POS	>4.9	2.9	4.8	3.7
13	POS	>4.9	>4.9	9.4	9.8
14	POS	>4.9	>4.9	8.0	7.7
15	POS	>4.9	4.4	8.7	7.3
16	POS	>4.9	>4.9	7.7	8.6
17	POS	>4.9	>4.9	8.8	12.0
18	POS	>4.9	>4.9	6.3	12.7
19	POS	>4.9	>4.9	6.1	11.3
20	POS	>4.9	>4.9	6.0	21.4
21	POS	>4.9	>4.9	5.9	21.4
22	POS	>4.9	>4.9	6.1	21.4
23	POS	>4.9	>4.9	6.4	16.9
24	POS	>4.9	>4.9	6.5	21.4
25	NEG	0.0	NEG	0.0	0.0

2. HCV genotype antibody testing

Genotype	Samples	Positive
1a-b	15	15
2a-b	13	13
3a-b	10	10
4h	6	6
5	12	12
6	18	18
Total	74	74

Sensitivity

Human Anti-Hepatitis C Virus Antibody ELISA Kit

Specificity

1. Clinical Specificity:

A blood donor population of 2948 individuals was tested with 3 different kits from different manufacturers. The specificity of this Anti-HCV ELISA was 99.55%

Manufacturers	-	+	False pos.	Specificity
Manufacturer 1	2896	38	14	99.52%
Manufacturer 2	2895	38	15	99.48%
Anti-HCV ELISA	2897	13	13	99.55%

2. Analytical Specificity:

No cross reactivity observed with samples from patients infected with HAV, HBV, HIV, CMV and TP. No interference was observed from rheumatoid factors up to 2000U/ml.

The performance characteristics of this assay are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein.

Same day and frozen specimens have been tested to check for interferences due to collection and storage.

Recovery

Reproducibility		Within run		Between run	
Sample	No.	Mean A value	CV%	Mean A value	CV%
Weak positive	10	0.436	9.1%	0.401	9.5%
Moderate positive	10	0.946	7.0%	0.916	7.5%
Strong positive 1	10	1.917	4.4%	1.895	4.2%
Strong positive 2	10	2.372	3.8%	2.309	4.0%

Precautions

TO BE USED ONLY FROM QUALIFIED PROFESSIONALS

The ELISA assays are time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. CAUTION - CRITICAL STEP: Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
7. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
8. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.
9. Assure that the incubation temperature is 37°C inside the incubator.



10. When adding specimens, do not touch the well's bottom with the pipette tip.
11. When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.
12. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.
13. If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.
14. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
15. **WARNING:** Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
16. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
17. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
18. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.
19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.
20. The Stop solution 0.5M H₂SO₄ is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.
21. ProClinTM 300 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

INDICATIONS OF INSTABILITY DETERIORATION OF THE REAGENT: Values of the Positive or Negative controls, which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested.

Limitations

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immunosuppressed individuals. Therefore, negative results obtained with this Anti-HCV ELISA are only indication that the sample does not contain detectable level of anti-HCV antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with HCV or the blood unit is not infected with HCV.

3. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as nonrepeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
4. The most common assay mistakes are: using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
5. The prevalence of the marker will affect the assay's predictive values.
6. This assay cannot be utilized to test pooled (mixed) plasma. This Anti-HCV ELISA has been evaluated only with individual serum or plasma specimens.
7. This Anti-HCV ELISA is a qualitative assay and the results cannot be use to measure antibodies concentrations.

