TRUSTwell®

HCV Total Ab ELISA Kit v.4

REF

AE0520

IVD

- 96-well ELISA kit for the qualitative detection of antibodies to Hepatitis C virus in human serum or plasma
- Store at 2-8 °C upon receipt

INTENDED USE

The TRUSTwell HCV Total Ab ELISA Kit is a solid-phase enzyme linked immunosorbent assay for the qualitative detection of antibodies (IgM, IgG & IgA) to Hepatitis C virus (HCV) in human serum or plasma. It is intended for professional use only as an aid in the diagnosis of infection with HCV. Any reactive specimen with the TRUSTwell HCV Total Ab ELISA Kit must be confirmed with alternative testing method(s) and clinicalfindings.

INTRODUCTION

Hepatitis C Virus (HCV) is a small, enveloped, positive-sense, single-stranded RNA Virus[1]. HCV is now known to be the major cause of the blood transmitted non-A, non-B hepatitis[2]. Antibodies to HCV are detectable about 45 days after exposed to HCV, and are found in over 80% of patients with well-documented non-A, non-B hepatitis. Therefore, detection of HCV antibodies in the serum or plasma is useful in the determination of HCV exposure and in the diagnosis of Hepatitis C[3,4].

The TRUSTwell HCV Total Ab ELISA Kit is the latest generation of solid phase enzyme linked immunoassay which specifically detects antibodies to HCV in human serum or plasma. The test is highly sensitive and specific.

TEST PRINCIPLE

The TRUSTwell HCV Total Ab ELISA Kit is a solid phase enzyme linked immunosorbent assay based on the principle of the sandwich EIA technique for the detection of the antibodies to HCV in human serum or plasma.

The TRUSTwell HCV Total Ab ELISA Kit is composed of two key components:

1) Solid microwells pre coated with recombinant HCV antigens(core-NS3-NS4-NS5)

2) Biotinylated HCV antigens

3) Liquid conjugates composed of streptavidin conjugated with horseradish peroxidase (SA-HRP conjugates).

During the assay, the test specimen and biotinylated HCV antigens are first incubated with the pre-coated microwells. The anti HCV, if present in the specimen, binds to the biotinylated antigens and forms a complex with coated microwell surface.

In the second incubation, the anti-HCV antibodies absorbed on the surface of microwell react to the streptavidin HRP conjugates.

Unbounded conjugates are then removed by washing. The presence of the complexed conjugates is shown by a blue color upon additional incubation with TMB substrate. The reaction is stopped with Stop Solution and absorbances are read using a spectrophotometer at 450 /620-690 nm.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	Microwells coated with recombinant HCV antigens	8 wells x 12 strips	AE0520W
2	HCV positive control	0.5 mL	AE0520P
3	HCV negative control	0.5 mL	AE0520N
4	Biotinylated HCV antigens (50x)	0.25 mL	AE0520B
5	Biotin diluent	12 mL	AE0520BD
6	SA HRP Enzyme Conjugate	12 mL	AE0520H
7	Wash buffer (30 X concentrate)	20 mL	AWE3000
8	TMB substrate A	6 mL	ATME2000A
9	TMB substrate B	6 mL	ATME2000B
10	Stop solution	12 mL	ASE1000
11	ELISA Working Sheet	2 Nos	AE0001ES
12	Product insert	1 No.	PI-AE0520

Materials and reagents required but not provided in the kit

1. Pipette capable of delivering 10 µL, 50 µL, and 100 µL volumes with a precision better than 1.5%.
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable.
3. Absorbent paper for blotting the microplate wells.
4. Parafilm or other adhesive film sealant for sealing plate.
5. Timer.
6. Distilled or de-ionized water.
7. Incubator

STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Store all components at 2-8°C. Do not freeze. Avoid strong light. Ensure that the reagents are brought to room temperature before opening. After removing the desired number of wells, place unused wells in the resealable plastic bag provided with desiccant and return to 2-8°C. All reagents are stable through the expiration date printed on the label if not opened. Once opened, the kit is stable for 8 weeks at 2-8°C, or until the labeled expiration date, whichever is earlier.

WARNING AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not use expired devices.
3. Bring all reagents to room temperature (18°C-28°C) before use.
4. Do not use the components in any other type of test kit as a substitute for the components in this kit.
5. Do not use hemolyzed blood specimen for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mucus. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens. Dispose of all specimens and materials used to perform the test as biohazardous waste.
9. In the beginning of each incubation and after adding Stopping Solution, gently rocking the microwells to ensure thorough mixing. Avoid the formation of air bubbles as which results in inaccurate absorbance values. Avoid splash liquid while rocking or shaking the wells
10. Don't allow the microplate to dry between the end of the washing operation and the reagent distribution.
11. The enzyme reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or substrate solution.
12. The substrate solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The Substrate B must be stored in the dark.
13. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
14. The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.
15. Avoid strong light during color development.

SPECIMEN COLLECTION AND PREPARATION

1. Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
2. This kit is designed for use with serum or plasma specimen without additives only.
3. If a specimen is not tested immediately, refrigerated at 2°C-8°C. If storage period greater than three days are anticipated, the specimen should be frozen (-20°C). Avoid repeated freezing-thawing of specimens. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
4. Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.
5. Do not use serum specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PREPARATION OF THE REAGENTS

1. Bring all reagents, controls to room temperature (18°C-28°C).
2. Dilute concentrated Wash Buffer 30 fold with water as following:

Plate	DI water	30 X wash buffer	Final volume
Full plate	580 mL	20 mL	600 mL
Half plate	290 mL	10 mL	300 mL
A quarter plate	145 mL	5 mL	150 mL

Warm up the concentrated Wash Buffer at 37°C to dissolve the precipitant if it appears.

3. Mix each reagent before adding to the test wells.
4. Determine the number of microwells needed and mark on the ELISA Working Sheet with the appropriate information. Positive and Negative Controls require to be run in duplicate to ensure accuracy.
5. Dilute the biotinylated HCV Ag 51 times (for example add 20 µL reagent to 1 mL biotin diluent). Mix the solution thoroughly before use.

No.of Strip	1	2	3	4	5	6	7	8	9	10	11	12
No.of Wells	8	16	24	32	40	48	56	64	72	80	88	96
Biotinlated HCV antigens(50X) - µL	20	40	60	80	100	120	140	160	180	200	220	240
Biotin Diluent (mL)	1	2	3	4	5	6	7	8	9	10	11	12

6. Determine the number of microwells needed and mark on the ELISA Working Sheet with the appropriate information. Positive and Negative Controls require to be run in duplicate to ensure accuracy.

ASSAY PROCEDURE

1. Remove the desired number of strips and secure them in the microwell frame. Reseal un-used strips.
2. Add specimens according to the designation on the ELISA Working Sheet
   - 2.1 Blank wells: Leave the blank well alone (2 wells). Don't add any reagents.
   - 2.2 Control and Test wells: Add 25 µL of Negative Control (2 wells), Positive control (2 wells), and 25 µL test specimen to all the wells (except Blank), then transfer 75 µL of biotinylated HCV antigens (diluted as per previous section) to all the wells (except Blank) respectively.

To ensure better precision, use pipette to handle solution.

3. Gently rock the plate wells for twenty seconds, then cover the plate with sealant.
4. Incubate the wells at 37°C for 60 minutes.
5. Carefully remove the incubation mixture by emptying the solution into a waste container. Fill each well with diluted wash buffer (350 µL per well) and shake gently for 20-30 seconds. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 4 more times.
6. Add 100 µL of SA HRP conjugates into each well except the blank well, cover the plate,
7. Incubate at 37°C for 30 minutes.
8. Wash the plate 5 times as step 5 described.
9. Add 50 µL of TMB substrate A & 50 µL of TMB substrate B into each well including the blank well.
10. Incubate at 37˚C in dark for 15 minutes.
11. Stop the reaction by adding 100 µL of stop solution to each well. Gently mix for 20 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
12. Set the microplate reader wavelength at 450nm and measure the absorbance (OD) of each well against the blank well within 15 minutes after adding Stop Solution. A filter of 620-690nm can be used as a reference wavelength to optimize the assay result.

Flow chart of assay procedure

1.	Secure strips in microwell frame	Number of strips
2.	Add Controls &specimens	25 µL
3.	Add Biotinylated HCV antigens & Gently rock	75 µL 20 seconds
4.	Incubate	37°C, 60 minutes
5.	Wash: manual or automatic	5 times
6.	Add SA HRP Enzyme Conjugate and Gently rock	100 µL 20 seconds
7.	Incubate	37°C, 30 minutes
8.	Wash: manual or automatic	5 times
9.	Add TMB substrate A & B and Gently rock	50 + 50 µL 20 seconds
10.	Incubate	37°C, 15 minutes
11.	Add Stop Solution. Gently rock	100 µL 20 seconds
12.	Read result	450/620-690 nm within 15 minutes

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cut-off value = **0.15 + NC**

NC: Mean OD of the negative control. Use 0.05 for calculation of the cut-off value if less than 0.05.

B. Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the Cut-off Value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cut-off Value}}$$

C. Assay validation

The mean OD value of the HCV Ab positive controls should be ≥ 0.50. The mean OD value of the HCV Ab negative controls should be ≤ 0.10.

If the above specifications are not met, the assay is invalid. Check the assay procedure including incubation time and temperature and repeat the assay.

A. Interpretation of the results

Specimen OD ratio

Negative	< 1.00
Positive	≥ 1.00

1. The negative result indicates that there is no detectable anti-HCV Ab in the specimen.
2. Results just below the cut-off value (Lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to retest in duplicate the corresponding specimens when it is applicable).
3. Specimens with cut-off ≥ 1.00 are initially considered to be positive by the TRUSTwell HCV Total Ab ELISA Kit. They should be retested in duplicate before final interpretation.

   If after re-testing of a specimen, the absorbance value of the 2 duplicates is less than the cut-off value, the initial result is non-repeatable and the specimen is considered to be negative with the TRUSTwell HCV Total Ab ELISA Kit.

   Non-repeatable reactions are often caused by:
   - Inadequate microwell washing,
   - Contamination of negative specimens by serum or plasma with a high antibody titer,
   - Contamination of the substrate solution by oxidizing agents (bleach, metal ions, etc.)
   - Contamination of the stopping solution

   If after retesting the absorbance of one of the duplicates is equal or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the TRUSTwell HCV Total Ab ELISA Kit, subject to the limitation of the procedure, described below.

PERFORMANCE CHARACTERISTICS

Clinical Performance

A total of 1110 patient specimens from susceptible subjects were tested by the TRUSTwell HCV Total Ab ELISA Kit. Comparison for all subjects is showed in the following table:

	TRUSTwell HCV Total Ab ELISA Kit		
Ref. EIA	**Positive**	**Negative**	**Total**
Positive	65	0	65
Negative	1	1044	1045
Total	66	1044	1110

Relative Sensitivity:100%, Relative Specificity:99.9 %, Overall Agreement:99.9 %

Precision

Intra-assay precision was determined by assaying 20 replicates of three negatives, three weak positives and three strong positives

Specimens	Number of specimens	No. of replicates	CV
Negatives	3	20	≤ 50 %
Strong positives	3	20	≤ 10 %
Weak positives	3	20	≤ 15 %

Cross-reactivity

No false-positive HCV Total Ab ELISA test results were observed on 10 positives specimens from each of the following disease states or special conditions, respectively: HIV/HBsAg/Syphilis/Dengue/Malaria/Typhoid

Interference

Common substances (such as pain and fever medication and blood components) may affect the performance of the TRUSTwell HCV Total Ab ELISA Kit. Interference was studied by spiking these substances into 3 clinical specimens: negative, HCV Ab low positive and high positive. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the TRUSTwell HCV Total Ab ELISA kit.

List of potentially interfering substances and concentrations tested:

1. Salicylic acid 4.34 mmol/L
2. Sodium citrate 1.3 %
3. Creatinine 442 µmol/L
4. EDTA 3.4 µmol/L
5. Glucose 55 mmol/L
6. Heparin 3,000 U/L
7. Bilirubin 10 mg/dL

EXTERNAL EVALUATION RESULTS

The TRUSTwell HCV Total Ab ELISA kit was externally evaluated by The National Institute of Biologicals and it complies with the CDSCO's specifications. The TRUSTwell HCV Total Ab ELISA kit qualified the evaluation with a sensitivity of 100% and a specificity of 100%.

LIMITATION OF THE TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of anti-HCV in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The TRUSTwell HCV Total Ab ELISA Kit is limited to the qualitative detection of anti-HCV in human serum or plasma. The intensity of the color does not have a linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates the absence of detectable anti-HCV. However, a negative test result does not preclude the possibility of exposure to or infection with HCV.
4. A negative result can occur if the quantity of anti-HCV present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a specimen is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. Choo, Q.L., G. Kuo, A.J. Weiner, L.R. Overby, D.W. Bradley, and M. Houghton. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989;244:359
2. Kuo, G., Q.L. Choo, H.J. Alter, and M. Houghton. An assay for circulating antibodies to a a major etiologic Virus of human non-A, non-B hepatitis. Science 1989; 244:362
3. Van der Poel, C. L., H.T.M. Cuypers, H.W. Reesink, and P.N.Lelie. Confirmation of hepatitis C Virus infection by new four-antigen recombinant immunoblot assay. Lancet 1991; 337:317
4. Wilber, J.C. Development and use of laboratory tests for hepatitis C infection:a review. J. Clin. Immunoassay 1993; 16:20

Index of Symbols

Consult instructions for use	REF Catalogue number	Use-by date
IVD For *in vitro* diagnostic use only	LOT Batch code	Tests per kit
Temperature limit 2-8°C	Do not re-use	Keep dry
Manufacturer	Date of manufacture	If device is non-sterile
Do not use if package is damaged	Keep away from sunlight	Warnings / Precautions

Athenese-Dx Pvt. Ltd.
Module No. 407 & 408, 4th Floor,
TICEL Bio Park II, No. 5, CSIR Road.
Taramani, Chennai-600113, India
Tel: +91-44-22541131
E-mail: info@athenesedx.com
Website: www.athenesedx.com

PI-AE0520 Rev. D
Effective date: 06.02.2024
English version