TRUSTwell®

HCV IgG ELISA Kit

REF

AE0510

IVD

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

INTENDED USE

The TRUSTwell HCV IgG ELISA Kit is a solid phase enzyme linked immunosorbent assay for the qualitative detection of IgG 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 IgG ELISA Kit must be confirmed with alternative testing method(s) and clinical findings.

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

TEST PRINCIPLE

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

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

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

2) Liquid conjugates composed of mouse anti-human IgG conjugated with horse radish peroxidase (HRP-anti Human IgG conjugates).

During the assay, the test specimen is first incubated with the coated microwells. The IgG anti HCV, if present in the specimen, bind to the antigens coated on the microwell surface.

In the second incubation with the HRP-anti-human IgG conjugates, the IgG antibodies absorbed on the surface of microwell react to the HRP-anti-human IgG 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	AE0510W
2	HCV positive control	0.5 mL	AE0510P
3	HCV negative control	0.5 mL	AE0510N
4	Sample diluent	12 mL	AE0510SD
5	HRP–anti human IgG conjugate	12 mL	AE0510H
6	Wash buffer (30 X concentrate)	20 mL	AWE3000
7	TMB substrate A	6 mL	ATME2000A
8	TMB substrate B	6 mL	ATME2000B
9	Stop solution	12 mL	ASE1000
10	ELISA Working Sheet	2 Nos	AE0001ES
11	Product insert	1 No.	PI-AE0510
12	Sealant	3 No.	N/A
13	Dessicant	4 No.	N/A

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. Return all reagents requiring refrigeration immediately after use. Reseal the microwells after removing the desired number of wells. Ensure that the reagents are brought to room temperature before opening. All the reagents are stable through the expiration date printed on the label if not opened. Do not freeze the kit or expose the kit over 8°C. 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 Kits.
3. Bring all reagents to room temperature (18-28°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 hemolized blood specimen for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mucose. 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.
9. Dispose of all specimens and materials used to perform the test as biohazardous waste.
10. 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 it may interfer in results. Avoid splash liquid while rocking or shaking the wells.
11. Don't allow the microplate to dry between the end of the washing operation and the reagent distribution.
12. The enzyme-substrate reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or TMB substrate solution.
13. The substrate solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The TMB Substrate B must be stored in the dark.
14. Use a new tip for each specimen. Never use the specimen container to dispense conjugate and substrate.
15. 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.
16. Avoid exposure of the well to 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 2-8°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 (18-28°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.

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 100 µL of Sample Diluent to all the wells (except Blank), then transfer 10 µL of each Positive control (2 wells), Negative Control (2 wells) and test specimen to each test well, respectively.

   To ensure better precision, use pipette to handle solution.

3. Gently shake the plate wells for 20 seconds then cover the plate with sealant.
4. Incubate the wells at 37°C for 30 minutes.

5. Carefully remove the incubation mixture by empting 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 HRP- anti-human IgG 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 and 50 µL of TMB substrate B into each well including the blank well.

10. Incubate at 37˚C in dark for 30 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 sample diluent	100 µL
3.	Add controls or specimens Gently rock	10 µL 20 seconds
4.	Incubate	37°C, 30 minutes
5.	Wash: manual or automatic	5 times
6.	Add Conjugate Gently rock	100 µL 20 seconds
7.	Incubate	37°C, 30 minutes
8.	Wash: manual or automatic	5 times
9.	Add TMB substrate A and B Gently rock	50 µL + 50 µL 20 seconds
10.	Incubate	37°C, 30 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 Blank should be ≤ 0.10.
The mean OD value of the HCV IgG positive controls should be ≥ 0.50.
The mean OD value of the HCV IgG negative controls should be ≤ 0.10.

D. Interpretation of the results

Specimen OD ratio

Negative	< 1.00
Positive	≥ 1.00

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

1. Specimen OD ratio ≥ 1.0 is considered as positive by the TRUSTwell HCV IgG ELISA Kit.
2. Specimen OD ratio < 1.0 is considered as negative result that indicates there is no detectable IgG anti-HCV in the specimen.
3. Specimen OD ratio of 0.9 - 1.1 should be interpreted with caution (it is advisable to re-test in duplicate with the corresponding specimens or re-test with fresh samples or advised to re-test with other confirmatory method(s)).
4. In cases where the initial and re-test results vary, the final interpretation should rely on the most reliable outcome.

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 stop solution.

PERFORMANCE CHARACTERISTICS

1. Clinical Performance

A total of 1108 patient specimens from susceptible subjects were tested by the TRUSTwell HCV IgG ELISA Kit. Comparison for all subjects is showed in the following table:

	TRUSTwell HCV IgG ELISA Kit		
Ref. EIA	**Positive**	**Negative**	**Total**
Positive	75	0	75
Negative	2	1031	1033
Total	77	1031	1108

Relative Sensitivity: 100%, Relative Specificity:99.81%,Overall Agreement:99.82%

2. 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	5.0 - 18.0 %
High positives	3	20	2.4 - 5.1 %
Low positives	3	20	4.6 – 9.4 %

3. Cross reactivity

No false positive HCV IgG 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

4. Interference

Common substances (such as painkiller, fever medication and blood components) may affect the performance of the TRUSTwell HCV IgG ELISA Kit. Interference was studied by spiking these substances into 3 clinical specimens: negative, HCV IgG 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 IgG 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

LIMITATION OF THE TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of IgG anti-HCV in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The TRUSTwell HCV IgG ELISA Kit is limited to the qualitative detection of IgG anti-HCV in human serum or plasma. The intensity of the color does not have linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable IgG 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 IgG 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
5. ISO 15223-1:2021 Medical devices — Symbols to be used with information to be supplied by the manufacturer — Part 1: General requirements.

Index of Symbols[5]

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