HCV Ab ELISA Kit

In vitro Diagnostics

CAT NO: S026-02

INTENDED USE

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

SUMMARY

Hepatitis C Virus (HCV) is a spherical enveloped, single-stranded positive sense RNA virus packed by core protein and enveloped by a lipid bilayer. HCV is the most common blood borne pathogen and a leading cause of morbidity and mortality. People infected with HCV are at a risk of developing chronic liver disease, cirrhosis and primary hepatocellular carcinoma. 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.

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

TEST PRINCIPLE

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

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

1) Solid microwells pre-coated with recombinant HCV antigen;
2) Liquid conjugates composed of mouse anti-human Ab conjugated with horse radish peroxidase (HRP-anti Human Ab conjugates).

During the assay, the test specimen is first incubated with the coated microwells. The Ab anti HCV, if present in the specimen, bind to the antigens coated on the microwell surface. In the second incubation with the enzyme conjugates, the Ab antibodies absorbed on the surface of microwell react to the HRP- anti-human Ab conjugates.

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

REAGENTS & MATERIALS PROVIDED

Item	Description	Quantity	Lot No:
1.	HCV Ag Coated Microwells	12 strips x 8 wells	MW001-03
2.	HCV Negative Control	0.5 mL	BS02702-01N
3.	HCV Positive Control	0.5 mL	BS02702-02P
4.	HCV Sample Diluent	12 mL	BS02602-04
5.	Enzyme Conjugate (HRP-anti human Ab conjugate)	12mL	BS02602-04
6.	Wash Buffer (30X)	20 mL	ER004-01
7.	TMB Substrate A	6 mL	ER005-01
8.	TMB Substrate B	6 mL	ER005-01
9.	Stop Solution	12 mL	ER006-01
10.	ELISA working sheet	2 Nos	ES001-01
11.	Product Insert	1 No	PIS02602-01

Materials and reagents required but not provided in the kit

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

STORAGE & STABILITY

Return all reagents requiring refrigeration immediately after use. All reagents except the concentrated wash buffer are ready to use as supplied. Reseal the microwells after removing the desired number of wells. All the reagents are stable through the expiration date printed on the label if not opened. Store the test kit over 8°C till the expiration date indicated on the pouch / carton. DO NOT FREEZE. Ensure that the test device is brought to room temperature before opening.

PRECAUTIONS & WARNING

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 hemolized blood specimen for testing.
5) Do not use the components in any other type of test kit as a substitute for the components in this kit.
6) 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
7) Don't allow the microplate to dry between the end of the washing operation and the reagent distribution.
8) 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.
9) Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
10) Users of this test should follow the US CDC's Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
11) Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
12) 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.
13) 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.
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.
16) Dispose of all specimens and materials used to perform the test as biohazardous waste

SAMPLE COLLECTION & 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.

REAGENT PREPARATION

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

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

If precipitant appears, warm up the concentrated wash buffer at 37°C.

3) Reagents should be mixed well before adding to the test wells.
4) Mark on the ELISA Working sheet with appropriate information after determining the number of microwells needed. 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 wells alone (2 wells). Don't add any reagents.
   - 2.2 Control and Test well: Add 100 µL of sample diluent to all the wells except (blank well), then transfer 10 µL of each positive control (2 wells), Negative control (2 wells) and test specimen to each test well respectively.
3) Cover the plate with sealant, rock the plate wells gently for 20 seconds.
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 second. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 4 more times.
6) Add 100 µL of HRP-antigen conjugate into each well except the blank wells, cover the plate.
7) Incubate the wells at 37°C for 30 minutes.
8) Wash the plate 5 times as described in step 5.
9) Add 50 µL of TMB substrate A and 50 µL of TMB substrate B into each well including the blank well.
10) Incubate the wells 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 20-30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

12) Set the microplate reader wavelength at 450 nm and measure the absorbance (OD) of each well against the blank well within 15 minutes after adding Stop Solution. A filter of 620–690 nm 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 specimen	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	450/620-690nm Within 15 minutes

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cutoff value = 0.15 + NC

NC: Mean OD value of Negative Control. Use 0.5 for the calculation of thecut-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 control should be ≥ 0.50

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

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

D. Interpretation of the results

Specimen OD ratio	
Negative	< 1.00
Positive	≥ 1.00

1) The negative result indicates that there is no detectable Ab anti-HCV 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 are initially considered to be positive by the Sensit HCV Ab ELISA kit. They should be retested in duplicate before the final interpretation.

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

Non repeatable reactions are often caused by:

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

If after re-testing 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 Sensit HCV Ab ELISA Kit, subject to the limitation of the procedure, described below.

PERFORMANCE CHARACTERISTICS

Clinical Performance

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

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%

Precision

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

Specimens	No. of Specimens	No. of replicates	CV
Negatives	3	20	5.6-18%
High Positives	3	20	2.4-5.1%
Low positives	3	20	4.6-9.4%

Cross-reactivity

No false positive HCV Ab ELISA test results were observed on 10 positive 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 Sensit HCV Ab ELISA Kit. Interference was studied by spiking these substances into 3 HCV clinical specimens: negative, low positive, and high positive. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the Sensit HCV Ab ELISA Kit.

List of potentially interfering substances and concentrations tested:

1. Salicylic acid 4.34mmol/L
2. EDTA 3.4 µmol/L
3. Glucose 55mmol/L
4. Sodium citrate 1.3%
5. Heparin 3,000 U/L

LIMITATION OF THE TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of Ab anti-HCV in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The Sensit HCV Ab ELISA Kit is limited to the qualitative detection of Ab anti-HCV in human serum or plasma. The intensity of color does not have linear correlation with the antigen titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable Ab 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 Ab 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

Key to symbols used			
	Manufacturer		Expiration/use by date
	Do not reuse		Date of manufacture
	Consult IFU [Instructions For Use]	LOT	Batch code
	Temperature limitation 2-30°C	IVD	In Vitro diagnostic medical device
	Contains sufficient for 'X ' kits		Do not use if package is damaged
	Keep dry	REF	Catalogue No

Manufactured by,

ubio Biotechnology Systems Pvt Ltd.
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