RecombiLISA

HCV Ab ELISA

IVD | REF E0511

- 96-well ELISA kit for the qualitative detection of antibodies to hepatitis C virus in human serum, plasma
- For export only, not for re-sale in the USA
- Store at 2-8°C upon receipt

INTENDED USE

The *RecombiLISA* HCV Ab ELISA is a solid-phase enzyme-linked immunosorbent assay for the qualitative detection of antibodies (IgG and IgM) 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.

INTRODUCTION

Hepatitis C Virus 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 *RecombiLISA* HCV Ab ELISA is a 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 *RecombiLISA* HCV Ab ELISA is a solid-phase enzyme-linked immunosorbent assay based on the principle of the indirect technique for the detection of anti-HCV antibodies (IgG and IgM) in human serum or plasma.

The *RecombiLISA* HCV Ab ELISA is composed of two key components:

1) Solid microwells pre-coated with recombinant HCV structural and non-structural antigens
2) Liquid conjugates composed of Protein A conjugated to horseradish peroxidase (HRP- Protein A Conjugate)

During the assay, the test specimen is first incubated in the coated microwells. The anti-HCV antibodies (IgG and/or IgM), if present in the specimen, bind to the antigens coated on the microwell surface, forming an immunocomplex. Any unbound materials are then removed by a wash step.

During the second incubation with the HRP-Protein A Conjugate, the protein A binds to the anti-HCV antibodies in the immunocomplex on the surface of the microwell.

Unbound materials are then removed by washing. After addition of the TMB substrate, the presence of the conjugate complex is shown by the development of a blue color resulting from a reaction between the enzyme and substrate. The reaction is then quenched by addition of the Stop Solution and the absorbance value for each microwell is determined using a spectrophotometer at 450 /620-690 nm.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	HCV Ag Coated Microwells	8 wells x 12 strips	E0511W
2	HCV Ab Positive Control	1 mL	E0511P
3	HCV Ab Negative Control	1 mL	E0511N
4	Sample Diluent	11 mL	E0511SD
5	HRP–Protein A Conjugate	11 mL	E0511H
6	Wash Buffer (30 x Concentrate)	20 mL	WE3000
7	TMB Substrate A	6 mL	TME2000A
8	TMB Substrate B	6 mL	TME2000B
9	Stop Solution	6 mL	SE1000
10	ELISA Working Sheet	2	E0001ES
11	Product insert	1	PI-E0511

Others 3 x Microplate Sealers and 1 x Resealable Bag

Materials and reagents required but not provided in the kit

1. Pipette capable of delivering 10 µL, 50 µL, and 100 µL
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
5. Timer
6. Distilled or de-ionized water

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. Reseal the microwells after removing the desired number of wells. Place unused wells in the resealable bag provided and return to 2-8°C. All the reagents are stable through the expiration date printed on the label if not opened.

SPECIMEN COLLECTION AND PREPARATION

- Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
- This kit is designed for use with serum or plasma specimen without additives only.
- If a specimen is not tested immediately, refrigerate at 2-8°C. If storage period greater than three days is 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.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.
- 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. **Preparation of working Wash Buffer.** Warm up the concentrated Wash Buffer to 37°C to dissolve the precipitant if it appears. Dilute concentrated Wash Buffer 30 fold with water as follows:

Plate	DI water	Wash buffer (30X)	Final volume
1 strip	58 mL	2.0 mL	60 mL
2 strips	116 mL	4.0 mL	120 mL
3 strips	174 mL	6.0 mL	180 mL
4 strips	232 mL	8.0 mL	240 mL

The diluted wash buffer can be stored at 2-8˚C for up to 3 days.

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 should be run in duplicate to ensure accuracy.

ASSAY PROCEDURE

1. Calculate the desired number of microwells. Remove the remaining microwells and place them with desiccant into the resealable plastic bag, seal and store at 2-8°C for later use.
2. Add specimens according to the designation on the ELISA Working Sheet:

2.1 Blank Well: Do not add any reagents.

2.2 Control Wells: Add 100 µL of Positive, Negative Control into the designated control wells, respectively.

2.3 Test wells: Add 100 µL of Sample Diluent to all the test wells, then transfer 10 µL of test specimens to each test well, respectively.

To ensure better precision, use pipette to handle solution.

3. Gently shake the plate for 20 seconds, then cover the plate with a sealer.
4. Incubate the microwells at 37°C for 30 minutes.
5. Wash Step (Can be performed manually or with automated washing): **Manual washing**: Carefully remove the incubation mixture by disposing the solution into a waste container. Fill each well with 350 µL of diluted Wash Buffer and rock gently for 20-30 seconds. Discard the wash solution completely. Repeat 4 more times. After completing the last wash step, tap the plate on absorbent paper to remove residual liquid.
Automatic washing: Automatic plate washer must be calibrated to ensure efficient washing. Aspirate incubation mixture from all wells completely. Fill each well with 350 µL diluted wash buffer and soak for 20-30 seconds. Aspirate all wells completely. Repeat 4 more times
6. Add 100 µL of HRP-Protein A Conjugate into each well except the Blank Well. Cover the plate with a sealer.
7. Incubate at 37°C for 20 minutes.
8. Wash the plate 5 times 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 at 37˚C in dark for 10 minutes.
11. Stop the reaction by adding 50 µL of Stop Solution to each well. Gently mix for 30 seconds. **It is important to make sure that all the blue color completely changes to a yellow color.**
12. Set the microplate reader wavelength at 450 nm. 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 controls into control wells; Add Sample Diluent and specimens to test wells, respectively	100 µL 100 µL + 10 µL
3.	Gently shake	20 seconds
4.	Incubate	37°C, 30 minutes
5.	Wash: manual or automatic	5 times
6.	Add HRP-Protein A Conjugate, except Blank Well	100 µL
7.	Incubate	37°C, 20 minutes
8.	Wash: manual or automatic	5 times
9.	Add TMB Substrate A and B, respectively	50 µL + 50 µL
10.	Incubate in dark	37°C, 10 minutes
11.	Add Stop Solution. Gently mix	50 µL, 30 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 + N

N: Mean OD of the negative control. Use N=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 ≥ 0.80.
The mean OD value of the HCV Ab negative controls should be ≤ 0.10.

Check the assay procedure including incubation time and temperature and repeat assay if above criteria is not met.

D. Interpretation of the results

	Specimen OD ratio
Negative	< 1.00
Positive	≥ 1.00

1. The negative result indicates that there are no detectable anti-HCV antibodies in the specimen.
2. Specimens with OD ratio ≥ 1.00 are initially considered to be positive by the *RecombiLISA* HCV Ab ELISA. They should be retested in duplicate before a final interpretation is made.
3. Results within 10% of the cut-off value should be interpreted with caution (it is advisable to re-test in duplicate the corresponding specimens when it is applicable).
   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 *RecombiLISA* HCV Ab ELISA, subject to the limitation of the procedure, described below.
   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 *RecombiLISA* HCV Ab ELISA.
   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 TMB Substrate by oxidizing agents (bleach, metal ions, etc.)
   - Contamination of the Stop Solution

PERFORMANCE CHARACTERISTICS

1. Clinical Performance

50 confirmed positive clinical specimens and 200 normal clinical specimens from susceptible subjects were tested by the *RecombiLISA* HCV Ab ELISA and by a Chinese State Drug Administration (CFDA) licensed reference EIA. Comparison for all subjects is showed in the following table:

	***RecombiLISA* HCV Ab ELISA**		
Ref. EIA	**Positive**	**Negative**	**Total**
Positive	50	0	50
Negative	0	200	200
Total	50	200	250

Relative Sensitivity: 100% (95% CI: 94.7% - 100%)
Relative Specificity: 100% (95% CI: 99.4% - 100%)
Overall Agreement: 100% (95% CI: 99.5% - 100%)

2. Cross-reactivity

No false positive *RecombiLISA* HCV Ab ELISA results were observed on 10 positive specimens from each of the following disease states or special conditions, respectively:

HBsAg HCV Syphilis Dengue Malaria Typhoid

3. Interference

Common substances (such as pain and fever medication and blood components) may affect the performance of the *RecombiLISA* HCV Ab ELISA. Interference was studied by spiking these substances into 3 levels of *RecombiLISA* HCV Ab ELISA 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 *RecombiLISA* HCV Ab ELISA.

List of potentially inferring substances and concentrations tested:

Potential Interfering Substance	Tested Concentration
Bilirubin	20 mg/dL
Glucose	55 mM
salicylic acid	4.34 mM
Sodium Citrate	3.8 %
EDTA	3.4 µM
Creatinine	442 µM
Heparin	3000 U/L

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 (18-28°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 mouth. 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 bio-hazardous waste.
10. In the beginning of each incubation and after adding Stop Solution, gently rock the microwells to ensure thorough mixing. Avoid the formation of air bubbles which results in inaccurate absorbance values. Avoid splashing liquid while rocking or shaking the wells
11. Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
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. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
15. The wash procedure is critical. Wells must be aspirated completely before adding the Wash Buffer or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.
16. Avoid strong light during color development.

LIMITATION OF THE TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of HCV Ab in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *RecombiLISA* HCV Ab ELISA is limited to the qualitative detection of anti-HCV antibodies 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 HCV Ab. 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 HCV Ab 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. If the symptom persists, while the result from *RecombiLISA* HCV Ab ELISA is negative or non-reactive result, it is recommended to test with an alternative method.
6. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
7. Any use or interpretation of this test's results must also rely on other clinical findings and the professional judgment of health care providers.

REFERENCES

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2. Kuo, G., Q.L. Choo, H.J. Alter, and M. Houghton. An assay for circulating antibodies to 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

See instructions for use		Use by	
IVD	For *in vitro* diagnostic use only	CONJ	Conjugate
REF	Catalog #	MICROWELLS	Coated microwells
LOT	Lot number	CONTROL +	Positive control
Σ N	Tests per kit	CONTROL -	Negative control
2	Do not reuse	SAMP DILT	Sample Diluent
	Manufacturer	TMB SUBS	TMB substrate
	Date of manufacture	WASH BUFF	Wash buffer
8°C 2°C	Store between 2-8°C	STOP SOLN	Stop solution

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