RecombiLISA

HCV IgG ELISA

IVD | **REF** **E0510**

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

INTENDED USE

The *RecombiLISA* HCV IgG ELISA is a solid-phase enzyme-linked immunosorbent assay for the qualitative detection of IgG 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.

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

TEST PRINCIPLE

The *RecombiLISA* HCV IgG ELISA is a solid-phase enzyme-linked immunosorbent assay based on the principle of the indirect technique for the detection of anti-HCV IgG antibodies in human serum or plasma.

The *RecombiLISA* HCV IgG ELISA is composed of two key components:
1) Solid microwells pre-coated with recombinant HCV antigens
2) Liquid conjugates composed of mouse anti-human IgG conjugated with horseradish peroxidase (HRP-anti Human IgG Conjugate)

During the assay, the test specimen is first incubated in the coated microwells. The anti-HCV IgG antibodies, if present in the specimen, bind to the antigens coated on the microwell surface, and any unbound material is then removed by a wash step.

During a second incubation with the HRP-anti-human IgG Conjugate, the anti-human IgG antibodies in the conjugate bind to the anti-HCV IgG antibodies bound to the surface of the microwell, forming a conjugate immunocomplex. Unbound materials are then removed by washing. After addition of the TMB substrate, the presence of the conjugate complex is shown by the presence 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	E0510W
2	HCV IgG Positive Control	1 mL	E0510P
3	HCV IgG Negative Control	1 mL	E0510N
4	Sample Diluent	11 mL	E0510SD
5	HRP-anti Human IgG Conjugate	11 mL	E0510H
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-E0510
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 450 nm wavelength is acceptable
3. Absorbent paper for blotting the microwells
4. Timer
5. 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, 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.
- 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 Strips 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. 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 HCV IgG 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 specimen to each test well, respectively.

3. Gently shake the microwells 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 shake 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 of diluted wash buffer and soak for 20-30 seconds. Aspirate all wells completely. Repeat 4 more times.

6. Add 100 µL of HRP-anti-human IgG 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 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 at 37˚C in dark for 10 minutes.
11. Stop the reaction by adding 50 µL of Stop Solution into each well. Gently mix for 30 seconds. **It is important to make sure that all the blue color completely changes to a color yellow.**
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. Add Sample Diluent and specimens	100 µL 100 µL + 10 µL
3. 4.	Gently shake Incubate	20 seconds 37°C, 30 minutes
5.	Wash: manual or automatic	5 times
6.	Add HRP-anti Human IgG Conjugate, except Blank Wells	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 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 IgG positive controls should be ≥ 0.80.
The mean OD value of the HCV IgG negative controls should be ≤ 0.10.
Check the procedure and repeat assay if above conditions are not met.

D. 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 IgG in the specimen.
2. Specimens with OD ratio ≥ 1.00 are initially considered to be positive by the *RecombiLISA* HCV IgG ELISA. They should be retested in duplicate before 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 to or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the *RecombiLISA* HCV IgG ELISA, subject to the limitations 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 IgG 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 solution by oxidizing agents (bleach, metal ions, etc.)
   - Contamination of the Stop Solution

PERFORMANCE CHARACTERISTICS

Clinical Performance

A total of 500 patient specimens were collected from susceptible subjects and tested by the *RecombiLISA* HCV IgG ELISA and by a Chinese State Drug Administration (SDA) licensed reference ELISA. Comparison for all subjects is showed in the following table:

	***RecombiLISA* HCV IgG ELISA**		
Ref. EIA	**Positive**	**Negative**	**Total**
Positive	48	0	48
Negative	0	452	452
Total	48	452	500

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

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. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
9. At the beginning of each incubation and after adding Stop Solution, gently rock the microwells to ensure thorough mixing. Avoid the formation of air bubbles as which results in inaccurate absorbance values. Avoid splashing liquid while rocking or shaking the wells.
10. Do not allow the microwells 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 TMB Substrate.
12. The TMB Substrate 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.
13. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and TMB Substrate.
15. The wash procedure is critical. Wells must be aspirated completely before adding the Washing Buffer or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance values. **The microplate reader must be calibrated as per manufacturer's instructions to ensure accurate determination of absorbance. A non-calibrated reader will often lead to invalid test results.**
16. Avoid exposure to 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 anti-HCV IgG in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *RecombiLISA* HCV IgG ELISA is limited to the qualitative detection of anti-HCV IgG 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 anti-HCV IgG. 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 IgG 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 results.
6. 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	Conjugates
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

CTK Biotech, Inc.
13855 Stowe Drive,
Poway, CA 92064, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

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