CE **RecombiLISA**

HEV IgM ELISA

IVD | **REF** **E0105**

- 96-well ELISA kit for the qualitative detection of anti-HEV IgM 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* HEV IgM ELISA is a solid-phase enzyme-linked immunosorbent assay for the qualitative detection of anti-hepatitis E virus (HEV) IgM in human serum or plasma. It is intended for professional use only and to be used as an aid in the diagnosis of infection with HEV.

INTRODUCTION

Hepatitis E, a major form of enterically transmitted hepatitis, is widespread in many developing countries but is currently considered an emerging threat to other parts of the world. HEV is a non-enveloped, positive-sense, single-stranded RNA virus[1,2]. It is currently classified within the family *Caliciviridae*. It is mainly transmitted through the fecal-oral route. At least four major genotypes of HEV have been recognized[3]: Genotypes 1 and 2 are restricted to humans while genotypes 3 and 4 can infect both humans and animals. Antibody response peaks approximately one month after initial infection. Antiviral IgM is detected in >90% patient and persists for 3 months. Anti-HEV IgM is also a well-established marker of recent infection[4] and is the most convenient one for diagnosis[5,6].

TEST PRINCIPLE

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

The *RecombiLISA* HEV IgM ELISA is composed of two key components:

1) Solid microwells pre-coated with polyclonal anti-human IgM antibody
2) Liquid conjugate composed of HEV antigen conjugated with horseradish peroxidase (HRP-HEV Conjugate)

During the assay, the test specimen is first incubated in the coated microwells. Anti-HEV IgM, if present in the specimen, binds to the antibody coated on the microwell surface. Any unbound specimen is then removed by a wash step.

During a second incubation with the HRP-HEV Conjugate, the anti-HEV IgM antibody is absorbed on the microwell surface which binds to the HRP-HEV Conjugate, forming a conjugate complex.

Unbound conjugate is then removed by washing. After addition of the TMB substrate, the presence of conjugate complex is shown by 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 test

Item	Description	Quantity	Catalog
1	Anti-human IgM Coated Microwells	12 wells x 8 strips	E0105W
2	HEV IgM Negative Control	0.5 mL	E0105N
3	HEV IgM Positive Control	0.5 mL	E0105P
4	Sample Diluent	11 mL	E0105SD
5	HRP-HEV Conjugate	11 mL	E0105H
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-E0105
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 50 µL and 100 µL volumes
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 water or de-ionized water

STORAGE AND STABILITY

All reagents with the exception of the concentrated wash buffer are ready for 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 plastic bag provided and return to 2-8°C. All 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, keep 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 precipitates 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 Washing Buffer:**
   If precipitants are visible, warm up the Wash Buffer (30X concentrate) at 37°C. 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

3. Mix each reagent before adding to the test wells.
4. Determine the number of microwells needed and mark the ELISA Working Sheet with appropriate information. Positive and Negative Controls need 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.

1. 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 HEV IgM Positive, Negative Control into the designated control wells, respectively.

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

To ensure better precision, use a pipette to handle solution.

2. Gently shake the microplate for 20 seconds, and cover the plate with a sealer.
3. Incubate the wells at 37°C for 30 minutes.
4. 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 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.

5. Add 100 µL of HRP-HEV conjugate into each well except the Blank Well, cover the plate with a sealer.
6. Incubate at 37°C for 30 minutes.
7. Wash the plate 5 times as described in step 5.
8. Add 50 µL of TMB Substrate A and 50 µL of TMB Substrate B into each well including the Blank Well.
9. Incubate at 37°C in the dark for 15 minutes.
10. Stop the reaction by adding 50 µL of Stop Solution into each well. Gently mix for 30 seconds. **It is important to make sure all the blue color completely changes to a color yellow.**
11. 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, respectively	100 µL
	Add Sample Diluent, then add specimen to test wells, respectively	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-HEV Conjugate, except Blank Well	100 µL
7.	Incubate	37°C, 30 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, 15 minutes
11.	Add Stop Solution.	50 µL
	Gently mix	30 seconds
12.	Read result	450/620-690 nm within 15 minutes

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cutoff value = 0.15+ N

N: Mean OD of the negative control. Use 0.05 for calculation of the Cut-off Value if the mean OD is 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-

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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 HEV IgM positive controls should be ≥ 0.80
The mean OD value of the HEV IgM 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-HEV IgM 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 applicable).
3. Specimens with OD ratio ≥ 1.00 are initially considered to be positive by the *RecombiLISA* HEV IgM ELISA. They should be retested in duplicate before final interpretation.
   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 positive with the *RecombiLISA* HEV IgM ELISA, subject to the limitation of the procedure, described below.
   If after re-testing of a specimen, the absorbance values 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* HEV IgM ELISA.
   Non-repeatable reactions are often caused by:
   - Inadequate microwell washing,
   - Contamination of negative specimens from 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

PERFORMANCE CHARACTERISTICS

1. Clinical Performance

A total of 400 specimens from susceptible subjects were tested by the *RecombiLISA* HEV IgM ELISA and by a Chinese State Drug Administration (SFDA) licensed reference EIA. Comparison for all subjects is showed in the following table:

	***RecombiLISA* HEV IgM ELISA**		
Ref. HEV IgM	**Positive**	**Negative**	**Total**
Positive	25	0	25
Negative	0	375	375
Total	25	375	400

Relative Sensitivity: 100%, Relative Specificity: 100%,
Overall Agreement: 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 serum derived from 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. 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 which results in inaccurate absorbance values. Avoid splashing liquid while rocking or shaking the wells.
11. Do not allow the microwells to dry between the end of the washing operation and reagent distribution.
12. The enzyme substrate reaction is very sensitive to metal ions. Thus, do not allow any metal elements to come into contact with the conjugate or TMB Substrate.
13. The enzyme-substrate is temperature dependent. Ensure that the room temperature for TMB incubation falls between 18-28°C.
14. 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.
15. Use a new dispensing tip for each specimen. Never use the specimen container for distribute conjugate and TMB Substrate.
16. **The wash procedure is critical. Wells must be aspirated completely before adding the Wash buffer or liquid reagents. Automatic washers must be validated with the test kit prior to use. Insufficient washing will result in poor precision and falsely elevated absorbance values.**
17. **Microplate reader must be calibrated per manufacturer's instruction to ensure accurate determination of absorbance. Non-calibrated reader often leads to invalid test results.**
18. 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 anti-HEV IgM in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *RecombiLISA* HEV IgM ELISA is limited to the qualitative detection of anti-HEV IgM in human serum or plasma. The intensity of 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-HEV IgM. However, a negative test result does not preclude the possibility of exposure to or infection with HEV.
4. A negative result can occur if the quantity of anti-HEV IgM present in the specimen is below the detection limit 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

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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
	Tests per kit	CONTROL -	Negative control
	Do not reuse	SAMP DILT	Sample diluent
	Manufacturer	TMB SUBS	TMB substrate
	Date of manufacture	WASH BUFF	Wash buffer
2°C 8°C	Store between 2-8°C	STOP SOLN	Stop solution

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