

Helicobacter Pylori Antibody IgM GENLISA® ELISA

REF : KBD932

Ver 1.4

IVD

Enzyme Immunoassay for the Qualitative Determination of Helicobacter
Pylori Antibody IgM in human serum and plasma

IVD	For In-vitro Diagnostic Use	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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REF KBD932 96 tests

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Introduction:

Helicobacter pylorus (*H. pylori*) is a gram negative bacterium which is normally found in gastric mucosa. The organism is present in 95-98% of patients with duodenal ulcer and 60-90% of patients with gastric ulcers. Estimation of infection rate by various diagnostic tests including bacteriological, histological and serological tests have revealed that 90% of symptomatic patients are affected and 50% of old age adults (> 50 years) is only colonized by bacteria lifelong without any clinical symptoms. Studies also have been demonstrated that removal of the organism by antimicrobial therapy is correlated with the resolution of symptoms and cure of diseases. Patients who present clinical symptoms relating to the gastrointestinal tract can be diagnosed for *H. pylori* infection by two methods:

1) Invasive techniques include biopsy followed by culture or histological examination of biopsy specimen or direct detection of urease activity.

2) Non-invasive techniques include urea breath tests and serological methods.

All of the tests performed on biopsy samples are subject to errors related to sampling and interference of bacterial contamination. *Helicobacter pylori* infection stimulates humoral immune response and provokes specific antibodies like IgG, IgM, and IgA. *H. pylori* IgM ELISA test is an accurate and simple technique to determine early stages of infection of bacteria and ELISA test is technique of choice for detection of IgM response.

Intended Use:

The *Helicobacter Pylori* Antibody IgM GENLISA® ELISA is intended for the qualitative determination of *Helicobacter Pylori* Antibody IgM in human serum and plasma.

Principle:

The *Helicobacter Pylori* Antibody IgM GENLISA® ELISA is an indirect enzyme linked immunosorbent assay for qualitative determination of IgM antibody present in the human serum and plasma. The Microtiter wells are pre-coated with specific *H. pylori* antigens. Samples and Controls are pipetted into microwells and *Helicobacter Pylori* Antibody IgM present in sample binds to the antigen coated on the wells. Anti-human-IgM:Enzyme Conjugate is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the substrate solution is added to microwells and color develops proportionally to the amount of *Helicobacter Pylori* Antibody IgM present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. *H. pylori* antigen Microtiter Coated Plate (96 wells) - 1 no
2. Positive Control – 1.5 ml
3. Negative Control – 2 ml
4. Sample diluent – 50 ml
5. Assay Buffer – 7.5 ml
6. Anti-human-IgM:Enzyme Conjugate – 12 ml
7. (10X) Wash Buffer – 2 x 50 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipette to measure volumes ranging from 25 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Timer.
7. Absorbent Paper.

Handling/Storage:

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.
4. Do not freeze.
5. Protect from light and moisture.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.



Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, re-centrifuge.

Sample Dilution:

To make 1:51 Dilutions, dilute 10 ul Sample + 500 ul Sample Diluent. Mix well

After that add 75 ul of Diluted sample with 75 ul of assay buffer and incubate for 20 min at room temperature.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 50ml of Wash Buffer (10X) to 450ml of DI water. This is the working solution.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **100 ul Controls and Diluted Sample** in appropriate wells.
3. Seal the plate and Incubate at 37°C for 30 minutes.
4. Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
5. Add **100 ul of Anti-human-IgM:Enzyme Conjugate** to each well.
6. Seal the plate and Incubate at 37°C for 30 minutes.
7. Repeat the Wash Step as mentioned in step 4.
8. Add **100 ul of TMB Substrate** into each well.
9. Incubate at room temperature for 15 minutes.
10. Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Calculation of Results:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Controls and Samples. Results are interpreted qualitatively by calculating a cut-off index for each sample on the basis of the cut-off determined.

Calculate Cut off value using following formula:

Cut off value = Mean of negative controls + 0.2

To determine positive and negative results calculate Cut off index using following formula:

Cut-off index (COI) = Sample OD/Cut-off value

Interpretation of Results:

Positive Sample:	Cut-off Index > 1.1
Equivocal Sample:	0.9 <= Cut-off index <= 1.1
Negative Sample:	Cut off index < 0.9

Control Criteria Validation:

Blank	O.D < 0.05
Negative Control	O.D < 0.1
Positive Control	O.D > 0.6

Performance Characteristics:

Sensitivity

The analytical sensitivity of **Helicobacter Pylori Antibody IgM** kit was calculated and determined to be **99.46%**

Specificity

The analytical specificity of **Helicobacter Pylori Antibody IgM** kit was calculated and determined to be **99.86%**

Precision:

To assess test precision, both intra-assay and inter-assay were run by positive, negative and three weekly positive samples.

Intra-Assay precision:

No.	Sample	No. of Replicates	OD Mean	SD	CV%
1	Positive control	20	1.51	0.071	4.7
2	Negative control	20	0.034	0.0022	6.5
3	Weakly positive sample 1	20	0.244	0.02	8.2
4	Weakly positive sample 2	20	0.314	0.019	6.0
5	Weakly positive sample 3	20	0.417	0.018	4.3

Inter-Assay precision:

No.	Sample	No. of Replicates	OD Mean	SD	CV%
1	Positive control	10	1.53	0.073	4.8
2	Negative control	10	0.034	0.0024	7.0
3	Weakly positive sample 1	10	0.25	0.021	8.4
4	Weakly positive sample 2	10	0.33	0.023	7.0
5	Weakly positive sample 3	10	0.43	0.025	5.8

*Each test has been run in duplicate

References

- Peterson W.L. (1991). H.pylori and peptic ulcer disease. N. Engl. J. Med. 324: 1024-1047
- M C Guigan J.E. (1988) Peptic ulcer and gastritis. Harrison's principles of internal medicine. 12th edition, chapter 238, 1229-1248.
- Podolsky I. (1989). Prevalence of H.pylori in healthy subject and patients with peptic disease. Gastroenterology 96 suppl. A394.
- C.I. Perez and M.O. Blaser (1991) Serodiagnosis of H.pylori: comparison of enzyme linked immunosorbent assay. H. Clin. Microbiol 29: 1635-1639.

Safety Precautions:

- This kit is For In-vitro Diagnostic Use only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed.
 - Do not smoke, eat or drink while handling kit material.
 - Always use protective gloves.
 - Never pipette material by mouth.
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.



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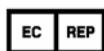
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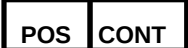
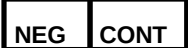
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SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add 100 ul Control, Diluted Sample in appropriate wells.
3	Seal the plate and Incubate at 37°C for 30 minutes.
4	Aspirate and wash plate 5 times with (1X) Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
5	Add 100 ul of Anti-human-IgM:Enzyme Conjugate to each well.
6	Seal the plate and Incubate at 37°C for 30 minutes.
7	Repeat the Wash Step as mentioned in step 4.
8	Add 100 ul of TMB Substrate into each well.
9	Incubate at room temperature for 15 minutes.
10	Add 100 ul of Stop Solution. Read result with an ELISA reader at 450 nm within 15 minutes stopping the reaction.

SYMBOLS KEY

	Microtiter Plate (96 wells)
	Positive Control
	Negative Control
	Sample Diluent
	Assay Buffer
	Anti-human-IgM:Enzyme Conjugate
	(10X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature