Helicobacter Pylori Antibody IgM GENLISA™ ELISA

REF : KBD932

Ver 1.1

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**

REF KBD932 Σ 96 tests

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Introduction:

Helicobacter pylori (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, histo-logical 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 immnunosorbent assay for qualitative determination of IgG 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 antibody 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 (8x12 wells) - 1 no
2. Positive Control – 1.5 ml/vial
3. Negative Control – 2 ml/vial
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 (20X) 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 except blank well.
6. 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.

Interpretation 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 value for each sample on the basis of the cut-off determined. Read Absorbance at 450nm with an ELISA reader.

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 by dividing sample

OD to cut off value:
Cut off index (COI) = OD of sample/Cutoff value

Positive Control:	Absorbance value > 1.1
Equivocal:	Absorbance value 0.9-1.1
Negative Control:	Absorbance value < 0.9

Criteria of Validation:

Blank	O.D < 0.05
Negative	O.D < 0.1
Positive Control	O.D > 1.0

Performance Characteristics:

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

Specificity

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

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	ODSD	CV%
1	Positive control	20	1.51	0.068	4.5
2	Negative control	20	0.03	0.002	6.0
3	Weakly positive sample 1	20	0.24	0.020	8.1
4	Weakly positive sample 2	20	0.30	0.020	6.7
5	Weakly positive sample 3	20	0.42	0.017	4.2

Inter-Assay precision:

No.	Sample	No. of Replicates	OD Mean	ODSD	CV%
1	Positive control	10	1.53	0.070	4.6
2	Negative control	10	0.03	0.002	6.2
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

1. Peterson W.L. (1991). H.pylori and peptic ulcer disease. N. Engl. J. Med. 324: 1024-1047
2. M C Guigan J.E. (1988) Peptic ulcer and gastritis. Harrison's principles of internal medicine. 12th edition, chapter 238, 1229-1248.
3. Podolsky I. (1989). Prevalence of H.pylori in healthy subject and patients with peptic disease. Gasteroentrology 96 suppl. A394.
4. 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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EC REP **KinesisDx, Lyoner Strasse 14, Frankfurt, Germany**

Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

** Under CDSCO Registration, please note*

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 except blank well.
6	**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 37°C for 10 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

MTP	Microtiter Plate (8x12 wells)
POS CONT	Positive Control
NEG CONT	Negative Control
SAMP DIL	Sample Diluent
ASSAY BUFF	Assay Buffer
ENZY CONJ	Anti-human-IgM:Enzyme Conjugate
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature