KRISHGEN PUDGALA
PARTNER FOR PRECISION DIAGNOSTICS

Helicobacter Pylori Antibody IgA GENLISA® ELISA

REF : KBD930

Ver 2.2

IVD

Enzyme Immunoassay for the Qualitative Determination of *Helicobacter Pylori* Antibody IgA 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 KBD930 Σ 96 tests

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Introduction:

Helicobacter pylori is a widespread microorganism with which the half of the world's population has been infected. Its prevalence is very high in developing countries and is quite low in the developed world. According to the World Gastroenterology Organization, the rate of infected adults in Eastern Europe and Asia is about 70-80%.
Studies during recent decades have shown the key role of bacterium *Helicobacter pylori* in the pathogenesis of stomach and duodenal lesions. *H. pylori* is detected almost in 100% of adult patients with duodenal ulcer, approximately in 80% of patients with peptic ulcer, in 92% of patients with gastric cancer and in 92% of patients with active chronic gastritis. Research has demonstrated that elimination of helicobacter leads to the disappearance of gastritis and significant reduction in the incidence of duodenal ulcer recurrence.
Helicobacteriosis is a chronic infection with long, often asymptomatic course. Its symptoms do not differ from clinical manifestations of gastro-duodenitis since usual constant pain in the epigastrium occurs. *H. pylori* is often present in patients with no clinical manifestations of disease.
H. pylori strains are extremely heterogeneous and divided into two groups: strains that express both antigens VacA and CagA (type I) and strains that do not express these antigens (type II). Strains of the first group dominate in patients with peptic ulcer disease and gastric cancer. CagA protein penetrates the epithelial cells of the mucous membrane, causes abnormal mitosis and induces chromosome instability. Organism infected with expressing CagA *H. pylori* produces antibodies, which are specific for CagA. Antibodies to CagA protein are found in 80-100% of patients with duodenal ulcer and in 94% of patients with gastric cancer. Thus, detection of the antibodies to CagA protein is an informative marker in the diagnosis of duodenal ulcer and gastric cancer.
Strains of *H. Pylori II* type, which do not express antigens CagA and VacA, are not associated with severe gastric and duodenal ulcers, particularly peptic ulcer disease and cancer.
H. pylori infection of the patient can be identified with both invasive and non-invasive diagnostic methods. Invasive methods include bioptic studies of the gastrointestinal tract mucosa with histological or culture methods or a rapid urease test. However, heterogeneous spread of *H. pylori* in tissues often leads to false-negative results. Non-invasive diagnostic methods include serologic studies of patient sera for the presence of specific antibodies to *H. pylori* and the urea breath test (UBT) with isotopically labeled urea. ELISA for the detection of specific IgG/IgA/IgM antibodies is the least invasive, rapid, highly sensitive and informative method for the diagnosis of *H. pylori* infection.
According to the research, a decrease in the titre of specific IgA antibodies is a reliable indicator of the effectiveness of treatment and eradication of *H. pylori*.

Intended Use:

The *Helicobacter pylori* Antibody IgA GENLISA® ELISA kit is used as an analytical tool for Qualitative determination of *Helicobacter pylori* Antibody IgA in serum, plasma and other biological samples.

Principle:

The method employs Indirect ELISA technique. Recombinant CagA protein of *H. pylori* is pre-coated onto microwells. Samples and standards are pipetted into microwells and *Helicobacter pylori* Antibody IgA present in the sample are bound by the antibodies. Anti-IgA conjugated to HRP is added and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of *Helicobacter pylori* Antibody IgA in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Recombinant CagA protein of *H. pylori* Coated Microtiter Plate (12 x 8 wells) – 1 no
2. Positive Control – 0.5 ml
3. Negative Control – 0.5 ml
4. Sample diluent – 12 ml
5. Anti-IgA:HRP Conjugate Solution – 12 ml
6. (20X) Wash Buffer – 50 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
9. 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 10 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.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. Wash Buffer (20X) Dilution: To make Wash Buffer (1X), add 4ml concentrate Wash Buffer (20X) + 76 ml of DI water is sufficient for 8 wells. Once diluted, it is stable at 2-8°C for 7 days. This is the working solution.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.

2. Add 9**0 ul Sample Diluent** into each well.

3. Add **10 ul** of **Negative controls**, **Positive Control** and **Samples** to respective wells.

4. Cover the plate with a sealer and incubate for 30 minutes at 37°C.

5. Aspirate and wash plate 4 times with diluted 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.

6. Add **100 ul** of **Anti-IgG:HRP Conjugate** to each well.

7. Cover the plate with a sealer and incubate for 30 minutes at 37°C.

8. Aspirate and wash as per step no. 5 above.
9. Add **100 ul** of **TMB Substrate** into each well.
10. Do not cover the plate with the sealer and incubate it in the dark for 15 minutes at 18-25°C.
11. Add 100 ul of Stop Solution to all wells. The wells should turn from blue to yellow in color.
12. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Validation of the test:

The test run may be considered valid provided the following criteria are met:

Positive Control	OD ≥ 1.200
Negative Control	OD ≤ 0.150
Negative Control	Nc x 0.5 ≤ Ncn ≤ Nc x 2.0, Ncn – each n-value of negatve control (Nc1, Nc2, Nc3)

If one of the negative control absorbance does not match the above criteria, this value should be discarded and a mean value should be calculated using the other two values. If more than one negative control absorbance does not meet the criteria, the test is invalid and must be repeated.

Calculation of Results:

Calculate the mean absorbance value for 3 negative controls (Nc), Cut off value (CO) and $Ratio_{sample}$

Nc = (Nc1 + Nc2 + Nc3) / 3;

CO = Nc + 0.3;

$Ratio_{sample} = OD_{sample}/CO$,

Interpretation of Results:

$Ratio_{sample}$ >1.1	Positive
0.9 ≤ $Ratio_{sample}$ ≤ 1.1	Doubtful
$Ratio_{sample}$ < 0.9	Negative

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

Interpretation of results of determination of IgG, IgA and IgM antibodies specific to *H. pylori* CagA protein

Specific antibodies to H. pylori CagA protein			Interpretation of result
IgG*	IgA*	IgM	
Negative	Negative	Negative	Sample does not contain specific antibodies to CagA *H. pylori*, or their concentration is below the sensitivity of the analysis.
Negative	Positive	Positive	Probable early stage of the infection, it is recommended to repeat the test in three weeks.
Negative	Positive	Negative	
Negative	Negative	Positive	
Positive	Negative	Negative	Sample contains specific antibodies to CagA protein of *H. pylori*, it is recommended additional tests: endoscopy, urease test, bacteriology.
Positive	Positive	Positive or Negative	

- The decrease of the specific IgG antibody titre is a reliable indicator of the efficacy of the treatment and eradication of *H. pylori*.

- At the *H. pylori* infection specific IgA antibodies are found in patients with cancer and stomach ulcers more often than in patients with chronic gastritis.

Performance Characteristics:

Specificity and Sensitivity
Sensitivity of the test kit *Helicobacter Pylori* Antibody IgA was evaluated using 82 sera which contain IgG antibodies to *H. pylori*. The sera were characterized in the CE-marked test kits for the determination of IgG antibodies to *H. pylori*. In the test kit *Helicobacter Pylori* Antibody IgA among IgG positive sera 22 samples (26.8%) were identified as positive (5).
In the study of 92 negative for antibodies to *H. pylori* sera, specificity rate was above 97.8%.

Precision

Intra-Assay precision:
Coefficient of variation (CV) was calculated by measuring 3 samples with various specific antibody levels in 32-replicate determinations using 1 lot of the test kit.

Serum No	OD_{mean}	$Ratio_{mean}$	CV%
849L	0.465	1.41	7.7
536	1.236	3.75	5.4
6s	0.613	1.86	6.4

Inter-Assay precision:
Coefficient of variation (CV) was calculated by measuring 3 samples with various specific antibody levels in 4 ELISA performances during 4 days, in 8-replicate determinations.

Serum No	OD_{mean}	$Ratio_{mean}$	CV%
863L	2.478	7.23	4.7
536	1.181	3.45	6.8
6s	0.599	1.75	7.1

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.

LIMITED WARRANTY

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India