Helicobacter pylori IgA ELISA Kit

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(Catalog # E4684-100, 96 assays; Store at 2-8^{0}C)

I. Introduction:

Helicobacter pylori (H. pylori) is detectable in nearly 100% of adult patients with duodenal ulcer and about 80% of patients with gastric ulcer. An association between H. pylori and gastric cancer is confirmed. In developing countries, where most children become infected by the age of 10, gastric cancer rates are very high. In the USA and other developed countries, standards of hygiene and the increasing socioeconomic status of the population have reduced the incidence of infection, and in parallel, the rates of peptic ulcers and gastric cancer have declined. There is excellent correlation between the clinical presentation of gastritis, the presence of H. pylori in the stomach and elevated serum H. pylori IgG and IgA antibodies. ELISA sensitivity and specificity are 90%, and the predictive value of a negative result for is very high. H. pylori IgG and/or IgA antibodies falls significantly after successful antibacterial therapy. Eradication of H. pylori is associated with a significant reduction in duodenal ulcer recurrence. pylori strains are classified into two broad groups - those that express both VacA and CagA (type I) and those that produce neither (type II). Type I strains are predominate in patients with ulcers and cancer. Up to 50% of adults is infected with H. pylori, but most of them are asymptotic and will not develop ulcer. The reason is they are infected with type II. 80-100% of patients with duodenal ulcer disease produce CagA antibodies against a 128 kd antigen compared with 60-63% of H. pylori-infected persons with gastritis only, indicating that serologic responses to the 128 kd protein are more prevalent among H. pylori-infected persons with duodenal ulcers than infected persons without peptic ulceration. In H. pylori-infected patients who develop gastric cancer, serum IgG against CagA 94% sensitive and 93% specific, indicating that detection of antibodies to CagA is useful marker for diagnosis of duodenal ulcer and gastric cancer.

II. Application:

Detection of IgA antibody to Helicobacter pylori

III. Sample Type:

Human serum or plasma

IV. Kit Contents:

Components	E4684-100	Part No.
Microplate	12 strips x 8 wells	E4684-100-1
Sample Diluent	22 ml	E4684-100-2
Calibrator	1 ml	E4684-100-3
Positive Control	1 ml	E4684-100-4
Negative Control	1 ml	E4684-100-5
Enzyme conjugate	12 ml	E4684-100-6
TMB Substrate	12 ml	E4684-100-7
Stop Solution	12 ml	E4684-100-8
Wash Buffer (20X)	25 ml	E4684-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VI. Storage Conditions and Reagent Preparation:

Store kit at 2-8^{0}C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

- **Wash Buffer:** Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

VII. Warning & Precautions:

- Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories."
- Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
- Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

VIII. Sample Preparation and Storage:

Collect blood specimens & separate the serum immediately. Specimens may be stored refrigerated at (2-8°C) for 7 days. Store frozen at (-20°C) for up to six month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well.

IX. Assay Protocol:

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use.

1. Place the desired no. of coated strips into the holder. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.

3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

X. Calculation

Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF). Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

EXAMPLE OF TYPICAL RESULTS:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

QUALITY CONTROL
The test run may be considered valid provided the following criteria are met:
1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should fall within the range specified on the COA/label.

INTERPRETATION
The following is intended as a guide to interpretation of IgA antibody test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

ANTIBODY INDEX INTERPRETATION

< 0.9	No detectable antibody to IgA antibody by ELISA.
0.9 - 1.1	Borderline positive. Follow-up testing is recommend if clinically indicated.
> 1.1	Detectable antibody to IgA antibody by ELISA.

LIMITATIONS OF THE TEST
1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.

Sensitivity and Specificity
94 sera from patients with suspected H. pylori infections were tested by this H. pylori IgA ELISA and a reference ELISA method. 14 sera were positive and 73 were negative by both methods (95% agreement). The results are summarized below:

		H. pylori IgA ELISA		
		+	–	Total
Reference ELISA Kit	+	14	3	17
	–	4	73	77
	Total	18	76	94

Precision
Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.43	0.096	6.71
2	16	0.98	0.067	6.83
3	16	0.26	0.019	7.30

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.29	0.121	9.37
2	10	0.91	0.089	9.78
3	10	0.23	0.025	10.86

XI. RELATED PRODUCTS:

- Helicobacter pylori IgG ELISA Kit (E4685)
- Helicobacter pylori IgM ELISA Kit (E4686)
- Steiner Stain Kit (K1435)
- QuickDetect™ IgA (Human) ELISA Kit (E4467)
- QuickDetect™ IgG (Human) ELISA Kit (E4475)
- QuickDetect™ IgM (Human) ELISA Kit (E4479)

FOR RESEARCH USE ONLY! Not to be used on humans.