

**Helicobacter pylori IgM
ELISA Kit Protocol**

(Cat. No.:EK-310-53)

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INTENDED USE

The Helicobacter pylori IgM Test Kit is intended for use in evaluating the serologic status to H. pylori infection in patients with gastrointestinal symptoms. **FOR RESEARCH ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES**

WARNINGS AND PRECAUTIONS FOR USERS

1. 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." 1984
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components from different lots should not be mixed.
4. 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.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

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INTRODUCTION AND PROTOCOL OVERVIEW

Helicobacter pylori is a spiral bacterium cultured from human gastric mucosa discovered by B.J. Marshall in 1982. Studies have indicated that the presence of H. pylori is associated with a variety of gastrointestinal diseases including gastritis, duodenal and gastric ulcers, non-ulcer dyspepsia, and gastric adenocarcinoma and lymphoma. The organism is present in 95-98% of patients with duodenal ulcers and 60-90% of patients with gastric ulcers. The studies have also demonstrated that removal of the organism by anti-microbial 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 histologic examination of biopsy specimen or direct detection of urease activity.
- (2) Non-invasive techniques – include urea breath tests and serological methods.

All of the testing performed on biopsy samples is subject to errors related to sampling and interference of contaminated bacteria. An ELISA test for the presence of H. pylori specific IgM antibody is the technique of choice for serologic tests because of its accuracy and simplicity.

PRINCIPLE OF THE TEST

Purified H. pylori antigen is coated on the surface of microwells. Diluted patients serum is added to the wells, and the H. pylori IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away. Enzyme conjugate is added, which binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

CAUTION: Phoenix Pharmaceuticals guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use and establish optimum sample concentrations.

STORAGE

Unopened test kits should be stored at 4°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement. **DO NOT FREEZE.**

LIST OF COMPONENTS

Materials Provided with the Kit:

- Purified *H. pylori* antigen coated microtiter plate, 96 wells.
- Enzyme Conjugate Reagent (red color), 13 ml.
- Sample Diluent (blue color), 22 ml.
- Negative Control, 150 µl.
- Cut-off Calibrator, *H. pylori* IgM EIA Index = 1, 150 µl.
- Positive Control, 150 µl.
- Wash Buffer (20X), 50 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials Required but not Provided:

- Distilled water.
- Precision pipettes: 5 µl, 100 µl and 200 µl.
- Disposable pipette tips.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.

SPECIMEN COLLECTION AND PREPARATION

1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.
2. Specimens may be refrigerated at 4°C for up to 7 days or frozen for up to 6 months. Avoid repetitive freezing and thawing of serum sample.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of Wash Buffer (1x). Wash buffer is stable for 1 month at 4°C. Mix well before use.

ASSAY PROCEDURE

Note: Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-25°C). **The wash procedure is critical. Insufficient washing will result in improper color development.**

1. Secure the desired number of coated wells in the holder.
2. Prepare 1:40 dilution of test samples, negative control, positive control, and calibrator by adding 5 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 for 10 seconds.
4. Incubate at room temperature for 30 minutes.
5. At the end of the incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 4 times with diluted wash buffer (1x) and then one time with distilled water. (Please do not use tap water.)
6. Dispense 100 µl of enzyme conjugate to each well. Mix gently for 10 second.
7. Incubate at room temperature for 30 minutes.
8. Remove enzyme conjugate from all wells. Rinse and flick the microtiter wells 4 times with diluted wash buffer (1x) and then one time with distilled water.
9. Add 100 µl of TMB Reagent to each well. Mix gently for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Add 100µl of Stop Solution to each well including the 2 blanks.
12. Mix gently for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read the optical density at 450 nm within 15 minutes with a microtiter plate reader.

CALCULATION OF RESULTS

1. Calculate the mean of duplicate calibrator value x_c .
2. Calculate the mean of duplicate positive control, negative control and patient samples.
3. Calculate the H. pylori IgM EIA Index of each determination by dividing the mean values of each sample by calibrator mean value, x_c .

Example of typical results: Note: The O.D. values are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data.

Cut-off Calibrator H. pylori IgM EIA Index = 1

1. Cut-off Calibrator O.D. = 0.650, 0.630 $x_c = 0.640$
2. Negative Control O.D. = 0.210, 0.230 $x_n = 0.220$
 H. pylori IgM EIA Index = $x_n / x_c = 0.220 / 0.640 = 0.340$
3. Positive Control O.D. = 1.105, 1.210 $x_p = 1.200$
 H. pylori IgM EIA Index = $x_p / x_c = 1.200 / 0.640 = 1.80$
4. Patient sample O.D. = 1.501, 1.670 $x_s = 1.600$
 H. pylori IgM EIA Index = $x_s / x_c = 1.600 / 0.640 = 2.50$

SUMMARY OF ASSAY PROCEDURE

1. Sample dilution 1:40
 5 μ l / 200 μ l
2. Three incubations at room temperature

Diluted Sample 100 μ l	Enzyme Conjugate 100 μ l	TMB Reagent (One-Step) 100 μ l
30 min.	30 min.	20 min

3. Stop with 100 μ l of acid. Read O.D. at 450 nm

QUALITY CONTROL

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

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.
2. If the O.D. value of the Cut-off Calibrator is lower than 0.250, the test is not valid and must be repeated.

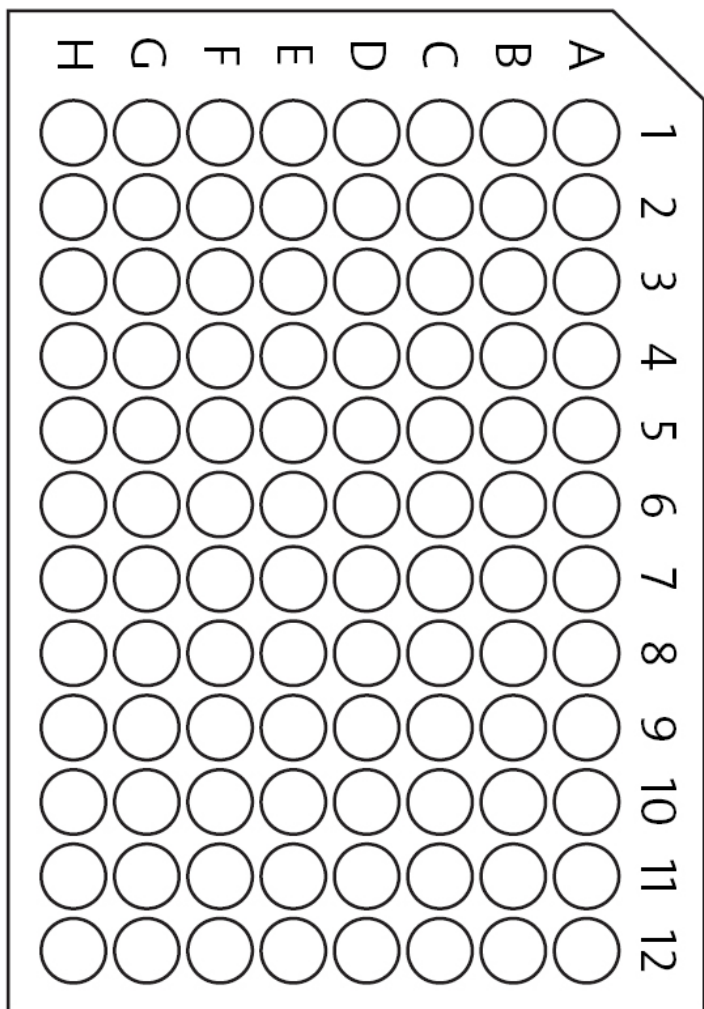
INTERPRETATION

- Negative: H. pylori IgM EIA Index less than 0.90 is seronegative for IgM antibody to H. pylori. The serum sample may have been taken too early.
- Equivocal: H. pylori IgM EIA Index between 0.91-0.99 is equivocal. Re-test In a parallel fashion with a new serum Sample drawn 3 weeks later.
- Positive: H. pylori IgM EIA Index of 1.00 or greater is seropositive.

REFERENCES

1. Marshall, B.J. and J.R. Warren. Unidentified curved bacilli in the stomach of patients with gastritis and Peptic ulceration. *Lancet* 1: 1311-1314, 1984.
2. Ruaws, E.A.J. and G.N.J. Tytgat. Cure of duodenal ulcer associated with eradication of *Helicobacter pylori*, *Lancet* 335: 1233-35, 1990.
3. Perez-Perez, G.I., S.S. Wilkin, M.D. Decker and M.J. Blaswer. Seroprevalence of *Helicobacter pylori* infection in couples. *J. Clin. Microbiol.* 29:642-644, 1991.

ASSAY DIAGRAM



NOTES