Product Description

*Helicobacter pylori* is detectable in nearly 100% of adult patients with duodenal ulcer and ~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 is very high. *H. pylori* IgG and/or IgA antibodies fall 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 are infected with *H. pylori*, but most of them are asymptotic and will not develop ulcers. The reason is they are infected with type II. 80–100% of patients with duodenal ulcer disease produce CagA antibodies against a 128 kDa antigen compared with 60–63% of *H. pylori*-infected persons with gastritis only, indicating that serologic responses to the 128 kDa 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 is 94% sensitive and 93% specific, indicating that detection of antibodies to CagA is a useful marker for diagnosis of duodenal ulcer and gastric cancer.

The *Helicobacter pylori* IgG ELISA Kit is intended for the detection of IgG antibody to *H. pylori* in human serum. Diluted serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the oxidation of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

Components

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwells coated with <em>H. pylori</em> antigen</td>
<td>12 x 8 x 1</td>
</tr>
<tr>
<td>Sample Diluent: 1 bottle (ready to use)</td>
<td>22 mL</td>
</tr>
<tr>
<td>Calibrator: 1 vial (ready to use)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Positive Control: 1 vial (ready to use)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Negative Control: 1 vial (ready to use)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Enzyme conjugate: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>TMB Substrate: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Stop Solution: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Wash concentrate 20x: 1 bottle</td>
<td>25 mL</td>
</tr>
</tbody>
</table>

Reagents and Equipment Required but Not Provided.

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.
Preparation Instructions
Sample Preparation
1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

20x Wash Buffer Concentrate
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).

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

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.

The test run may be considered valid provided the following criteria are met:
1. The O.D. of the Calibrator should be >0.250.
2. The Ab index for Negative control should be <0.9.
3. The Ab Index for Positive control should be >1.2.

Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 21-fold 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 of 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 absorbent 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 absorbent paper or paper towel.
7. Dispense 100 µL of TMB substrate and incubate for 10 minutes at room temperature. Add 100 µL of stop solution.
8. Read O.D. at 450 nm using ELISA reader within 15 minutes. A dual wavelength is recommended with reference filter of 600–650 nm.
**Results**

**Calculations**

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. Calculate the cut-off value: Calibrator OD × Calibrator Factor (CF).
3. 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 × 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

**Note:** 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. Lipemic or hemolyzed samples may cause erroneous results.

**Interpretation**
The following is intended as a guide to interpretation of *H. pylori* IgG antibody index (Ab Index) test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

<0.9 – No detectable antibody to *H. pylori* IgG by ELISA
0.9–1.1 – Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1 – Detectable antibody to *H. pylori* IgG by ELISA

**References**