The H. pylori IgG EIA Test Kit is an enzyme immunoassay for the qualitative and quantitative detection of IgG antibodies to H. pylori in human serum or plasma. It is intended as an aid in the diagnosis of possible H. pylori infection.

## INTENDED USE

- ProClin™ 300 is included as a preservative in the Conjugate, Concentrated Wash Buffer, Specimen Diluent, Substrate and Calibrators. Avoid any contact with skin or eyes.
- Do not eat, drink or smoke in the area where the kits or products are handled. Do not pipeet by mouth.
- Avoid any contact of the Substrate and Stop Solution with skin or mucosa. The Stop Solution contains sulfonic acid which is a strong acid. If spills occur, wipe immediately with large amounts of water. If the acid contacts the skin or eyes, flush with large amounts of water and seek medical attention.
- Do not dispose of apparatus should be sterilized after use. Do not autoclave materials containing sodium hydroxide.
- Handle and dispose all specimens and materials used to perform the test as if they contained infectious agents. The test results are to be interpreted by following all local, state and federal regulations.
- Neutralized antibodies should be determined by adding sufficient volume of sodium hydroxide to obtain a final concentration of at least 1.0%. A 30 minute exposure to 1.0% sodium hydroxide may be necessary to ensure effective decontamination.

## SUMMARY

- H. pylori is a small, spiral-shaped bacterium that lives in the surface of the stomach and duodenum. It is associated in the etiology of a variety of gastrointestinal diseases including duodenal and gastric ulcer, duodenal ulcers, 80% of gastric ulcers, and 70% of gastritis. Recently, it has been classified as a Class I carcinogen by the WHO. Although the transmission route for H. pylori is not yet known, it is believed to be transmitted by oral-oral or fecal-oral route. Both invasive and non-invasive methods are used to diagnose H. pylori infection in gastrointestinal disease. Invasive and non-invasive diagnostic methods include gastric or duodenal biopsy followed by urease testing (presumptive), culture, and/or histologic staining. Non-invasive tests include the breath test and others that use expensive laboratory equipment and moderate radiation exposure, and serological methods. Individuals infected with H. pylori may experience non-specific symptoms such as epigastric pain, indigestion, anorexia, nausea, and bile reflux, which are not specific to H. pylori infection. Early in the course of active infection, IgG antibody levels may be detectable. Levels of IgG antibody rise and remain constant for up to 7 years after infection. Therefore, the effect of therapeutic intervention may be assessed only after a significant delay.

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## STABILITY

- Unopened test kits should be stored at 2-8°C upon receipt. All reagents are stable through the expiration date printed on the box if stored between 2-8°C. Once opened, all reagents should be stored at 2-8°C. Do not store Calibrator in Liquid form at room temperature.
- The H. pylori IgG EIA Test Kit is a solid phase enzyme immunoassay based on indirect principle for the qualitative and quantitative detection of IgG antibodies to H. pylori in human serum or plasma. The microwell plate is coated with recombinant antigens specific for H. pylori and the specimens are added to the antigen coated microwell plate and then incubated. If the specimens contain specific antibodies the antibodies will bind to the immobilized antigen. After incubation, the microwell plate is washed to remove unbound materials. The enzyme-conjugated anti-human IgG antibodies are added to the microwell plate and then incubated. The enzyme-conjugated anti-human IgG antibodies will bind to the immobilized antigen and the antigen-antibody complexes are washed to remove unbound materials. Materials A and B are added and then incubated. The amount of H. pylori IgG antibodies present in the specimens, is measured with a microplate reader at 405/630-700 nm or 405 nm.

## PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after expiration date.
- Do not mix reagents from other kits with different lot numbers.
- Avoid cross-contamination between reagents to avoid test results.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer during incubation to prevent evaporation.
- Use a new pipet tip for each specimen assayed.
- Ensure that the bottom of the wells is visible and that no bubbles are present on the surface of the liquid before reading the plate. Do not allow wells to dry out during the assay procedure.
- Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with your finger.
- Do not allow sodium hydroxide fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer’s instructions.

## ADDITIONAL SAFETY INFORMATION

- Some components of this kit contain human blood derivatives. No known test method can offer complete assurance that products derived from human blood will not transfuse agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
- Wear disposable protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.

## DIRECTIONS FOR USE

- Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the contents of the bottle containing the concentrated wash buffer into a graduated cylinder and fill it with distilled water until the volume reaches 1,990 mL. Store the concentrated wash buffer and working wash buffer at 8°C.
- Add 100 μL of Calibrator 1 in wells B1 and C1. (Yellow Reagent)
- Add 100 μL of Calibrator 2 in wells D1 and E1: Add 100 μL of Calibrator 3 in wells F1 and G1. (Green Reagent)
- Add 100 μL of Calibrator 4 in wells h1 and A2. (Blue Reagent)
- Add 100 μL of Calibrator 5 in wells B2 and C2. (Red Reagent)
- Add 100 μL of Calibrator 6 in wells D2 and E2. (Orange Reagent)
- Add 100 μL of Specimen Diluent to assigned wells starting at F2. (Green Reagent)
- Add 100 μL of Specimen to assigned wells starting at F2. (Green Reagent)
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Absorbance of Calibrator 2. See an example of Cut-Off calculation below.

\[
\text{Cut-Off Value} = \text{Mean Absorbance of Calibrator 2} - \text{Blank Absorbance}
\]

\[
\text{Cut-Off Value} = 0.436 - 0.014 = 0.422
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Draw the calibration curve and obtain absorbance results for each specimen.

1. Subtract the Blank Absorbance from the Mean Absorbance of each Calibrator, then plot them on the Y-axis against their concentration in AU/mL, on the X-axis of the graph paper and draw the calibration curve. Draw the best fitted line through the data points to obtain a standard curve. Refer to an example of the calibration curve at right.

2. Obtain quantitative results from the absorbance obtained using the calibration curve.

**NOTE:** Use the calibration curve at right to make any further calculation. A calibration curve must be performed for each run.

**PERFORMANCE CHARACTERISTICS**

**1. Sensitivity and Specificity**

The H. pylori IgG EIA Test Kit has been compared to a leading commercial H. pylori IgG EIA test using clinical specimens. The results show that the clinical sensitivity of the H. pylori IgG EIA Test Kit is 97.9%, and the clinical specificity is >99.9%.

**2. Reproducibility**

Within-run precision has been determined by using 10 replicates of three specimens: a low positive, a medium positive and a high positive. Inter-Assay: Between-run precision has been determined by 3 independent assays on the same three specimens: a low positive, a medium positive and a high positive. Three different lots of the H. pylori IgG EIA Test Kit have been used for these specimens.

**BIBLIOGRAPHY**


