Foresight®

H. pylori Antigen EIA Test Kit
Package Insert

REF I231-1231 | English

An enzyme immunoassay (EIA) for the qualitative and quantitative detection of Helicobacter pylori (H. pylori) Antigen in human stool.

For professional in vitro diagnostic use only.

INTENDED USE

The *H. pylori* Antigen EIA Test Kit is an enzyme immunoassay for the qualitative and quantitative detection of *H. pylori* antigen in human stool. It is intended as an aid in the diagnosis of possible *H. pylori* infection and in the follow-up of patients undergoing antimicrobial therapy.

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, non-ulcer dyspepsia and active and chronic gastritis.[1,2] *H. pylori* infection is present in over 90% of duodenal ulcers, 80% of gastric ulcers, and 70% of gastritis. The transmission route for *H. pylori* is currently unknown but believed to be transmitted by oral-oral or fecal-oral route.

Methods of diagnosing *H. pylori* infection have been developed along two lines including the direct detection of the organism and the detection of antibodies to *H. pylori*. The direct, invasive and costly methods include urease testing (presumptive), culture, and/or histologic staining of biopsy material. Another direct method is the urea breath test, though non-invasive and highly sensitive and specific, requires expensive laboratory equipment and moderate radiation exposure. The most common non-invasive method is the serological detection of antibodies specific to *H. pylori.* Individuals infected with *H. pylori* develop serum antibodies which correlate strongly with histologically confirmed *H. pylori* infection.[3,4,5] Early in the course of active infection, IgM antibody levels are detectable followed by a rise of IgG and IgA antibodies which remain constantly high until infection is eliminated. More recently, the use of enzyme immunoassay to detect the *H. pylori* antigens, which illicit the immune response, has been used for diagnosis of active *H. pylori* infection, patient monitoring after eradication therapy or reinfection.

The *H. pylori* Antigen EIA Test Kit is an immunoassay for the qualitative and quantitative detection of the presence of *H. pylori* Antigen in human stool. The test utilizes antibodies to *H. pylori* to selectively detect *H. pylori* Antigen in human stool.

PRINCIPLE

The *H. pylori* Antigen EIA Test Kit is a solid phase enzyme immunoassay based on sandwich principle for the qualitative and quantitative detection of *H. pylori* antigen in human stool. The microwell plate is coated with anti-*H. pylori* antibodies. During testing, the antigens are extracted out with extraction solution and added to the antibodies coated microwell plate with the enzyme-conjugated antibodies to *H. pylori* and then incubated. If the specimens contain *H. pylori* antigens, it will bind to the antibodies coated on the microwell plate and simultaneously bind to the conjugate to form immobilized antibody-*H. pylori* antigen-conjugate complexes. If the specimens do not contain *H. pylori* antigens, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of *H. pylori* antigens present in the specimens. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of *H. pylori* antigens present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 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 ensure valid test results.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipet tip for each specimen assayed.
- Ensure that the bottom of the plate is clean and dry 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 fingertips.
- Do not allow sodium hypochlorite 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.

HEALTH AND SAFETY INFORMATION

- Human specimens should be considered potentially hazardous and handled using established good laboratory working practices.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.
- ProClin™ 300 is included as a preservative in the Conjugate, Concentrated Wash Buffer, Extraction Solution, Substrate and Calibrators. Avoid any contact with skin or eyes.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not pipette by mouth.
- Avoid any contact of the Substrate and Stop Solution with skin or mucosa. The Stop Solution contains 0.5M sulfuric 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.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- Handle and dispose all specimens and materials used to perform the test as if they contained infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.
- Observe Good Laboratory Practices when handling chemicals and potentially infectious material. Discard all contaminated material, specimens and reagents of human origin after proper decontamination and by following local, state and federal regulations.
- Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

STORAGE AND STABILITY

- Unopened test kits should be stored at 2-8°C upon receipt. All unopened reagents are stable through the expiration date printed on the box if stored between 2-8°C. Once opened, all reagents are stable for up to 3 months after the first opening date if stored between 2-8°C. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch and remove the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch with desiccant supplied at 2-8°C and can be used within 3 months of the opening date. Return the remaining unused strips and supplied desiccant to the original resealable pouch, firmly press the seal closure to seal the pouch completely and immediately store at 2-8°C.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
- Do not store Stop Solution in a shallow dish or return it to the original bottle after use.

SPECIMEN COLLECTION AND PREPARATION

- The *H. pylori* Antigen EIA Test Kit can be performed using only human stool.
- The patient has to be asked to collect the specimen avoiding any possible contact with urine or water.
- The patient submitted to the test should not be under antibiotic or anti-bacterial treatments as this pharmaceutical therapy is known to affect *H. pylori* up to a certain extent, depending on the antibiotic used, giving origin to false interpretation.
- Stools may be stored at 2-8°C for up to 24 hours prior to assaying. For long term storage, specimens should be kept frozen below -20°C. Degradation of *H. pylori* antigen heavily occurs in stool after 24 hrs generating false negative results, even if the specimen is stored at 2-8°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

REAGENTS AND COMPONENTS

Materials Provided

No.	Reagent	Component Description	Quantity	
			96 wells/kit	480 wells/kit
	H. pylori Antigen Microwell Plate	Microwell plate coated with anti-*H. pylori* antibodies	1 plate (96 wells/ plate)	5 plates (96 wells/ plate)
1	*H. pylori* Antigen Conjugate	Antibodies to *H. pylori* bound to peroxidase; Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL
2	Concentrated Wash Buffer (25x)	Tris-HCl buffer containing 0.1% Tween20; Preservative: 0.1% ProClin™ 300	1 x 40 mL	5 x 40 mL
2A	Extraction Solution	0.9% NaCl buffer containing EDTA; Preservative: 0.1% ProClin™ 300	2 x 50 mL	10 x 50 mL
3	Substrate A	Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL
4	Substrate B	Buffer containing tetramethylbenzidine (TMB); Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL
5	Stop Solution	0.5M Sulfuric acid	1 x 8 mL	5 x 8 mL
6	*H. pylori* Antigen Calibrator 1	Buffer non-reactive for *H. pylori* Antigen; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL
7	*H. pylori* Antigen Calibrator 2	Buffer containing 0.1 µg/mL *H. pylori* Antigen; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL
8	*H. pylori* Antigen Calibrator 3	Buffer containing 0.5 µg/mL *H. pylori* Antigen; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL
9	*H. pylori* Antigen Calibrator 4	Buffer containing 1.0 µg/mL *H. pylori* Antigen; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL
	Specimen Extraction Kit*	Each kit contains 48 Specimen Extraction Tubes, and 30 Liquid Specimen Droppers	2 x 1 kit	10 x 1 kit
	Plate Sealers		2	10
	Package Insert		1	1

* Specimen Extraction Kit is packaged separately.

Materials Required But Not Provided

- Freshly distilled or deionized water
- Sodium hypochlorite solution for decontamination
- Absorbent paper or paper towel
- Water bath or incubator capable of maintaining 15°C to 30°C.
- Calibrated automatic or manual microwell plate washer capable of aspirating and dispensing 350 µL/well
- Disposable gloves
- Automated processor (optional)
- Calibrated micropipettes with disposable tips capable of dispensing 50 and 100 µL
- Graduated cylinders for wash buffer dilution
- Vortex mixer for specimen mixing (optional)
- Disposable reagent reservoirs
- Calibrated microplate reader capable of reading at 450 nm with a 630-700 nm reference filter, or reading at 450 nm without a reference filter
- Timer

DIRECTIONS FOR USE

Allow reagents and specimens to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange the calibrators so that well A1 is the Blank well. From well A1, arrange the calibrators in a horizontal or vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.

Step	Detailed Procedure	Simplified Procedure
	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the contents of the bottle containing the concentrated wash buffer in a graduated cylinder and fill it with freshly distilled or deionized water to 1000 mL for 96 wells/plate testing. The Working Wash Buffer is stable for 2 weeks at 15-30°C. **Note:** If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C until all crystals dissolve. • Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25 • Remove and store unused strips at 2-8°C
	• Transfer 1 mL of Extraction Solution into Specimen Extraction Tube. • **<u>For Solid Stool Specimens</u>**: Unscrew the cap of the Specimen ExtractionTube, then randomly stab the specimen collection stick into the stool specimen in at least 3 different sites to collect approximately 30 mg of specimen (equivalent to 1/4 of a pea). Do not scoop the stool specimen. Then transfer into Specimen Extraction Tube. **<u>For Liquid Stool Specimens</u>**: Hold the Liquid Specimen Dropper vertically, aspirate stool specimens and then transfer 2 drops (approximately 50 µL) into the Specimen Extraction Tube containing the Extraction Solution. Screw on and tighten the cap onto the Specimen Extraction Tube, then shake the Specimen Extraction Tube vigorously to mix the specimen and the Extraction Solution.	• Transfer 1 mL of extraction solution into Specimen Collection Tube. • Transfer 30 mg solid specimen or 50 µL liquid specimens to the Specimen Extraction Tube. Mix and vortex.
0	• Leave A1 as Blank well.	• Leave A1 as Blank well

1	• Add 50 µL of Calibrator 1 in wells B1 and C1. (Yellow Reagent) • Add 50 µL of Calibrator 2 in wells D1 and E1. (Blue Reagent) • Add 50 µL of Calibrator 3 in wells F1 and G1. (Blue Reagent) • Add 50 µL of Calibrator 4 in wells H1 and A2. (Blue Reagent)	• B1 and C1: Add 50 µL Calibrator 1 • D1 and E1: Add 50 µL Calibrator 2 • F1 and G1: Add 50 µL Calibrator 3 • H1 and A2: Add 50 µL Calibrator 4
2	• Hold the Specimen Extraction Tube upright and break off the tip of the tube. Invert the Specimen Extraction Tube and transfer 2 drops of the specimen Extraction Solution (approx. 50 µL) to assigned wells starting at B2. (Yellow Reagent) • Add 50 µL of Conjugate to each well except for the Blank well. (Red Reagent)	• Starting B2: Add 2 drops of the specimen Extraction Solution • Add 50 µL of Conjugate to each well except for the Blank well
3	• Mix gently by swirling the microwell plate on a flat bench for 30 seconds. • Cover the microwell plate with the Plate Sealer and incubate at room temperature (15-30°C) in a room, a water bath, or an incubator for 60 minutes ± 5 minutes.	• Mix gently • Cover the microwell plate with the Plate Sealer and incubate at room temperature for 60 min
4	• Remove the Plate Sealer. • Wash each well 5 times with 350 µL of Working Wash Buffer per well, then remove the liquid. • Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried. Note: Improper washing may cause false positive results.	• Remove the Plate Sealer • Wash each well 5 times with 350 µL of Working Wash Buffer • Turn the microwell plate upside down on absorbent tissue
5	• Add 50 µL of Substrate A to each well. (Clear Reagent) • Add 50 µL of Substrate B to each well. (Clear Reagent) Then a blue color should develop in wells containing Positive specimens.	• Add 50 µL of Substrate A to each well • Add 50 µL of Substrate B to each well
6	• Mix gently then cover microwell plate with Plate Sealer and incubate at room temperature (15-30°C) in a room, a water bath, or an incubator for 10 minutes ± 1 minute.	• Mix then cover microwell plate with Plate Sealer and incubate at room temperature for 10 min
7	• Remove the Plate Sealer. • Add 50 µL of Stop Solution to each well. (Clear Reagent) Then a yellow color should develop in wells containing Positive specimens.	• Remove Plate Sealer • Add 50 µL of Stop Solution to each well
8	• Read at 450/630-700 nm within 30 minutes. Note: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450/630-700 nm for better results.	• Read at 450/630-700 nm within 30 min

VALIDATION REQUIREMENT AND QUALITY CONTROL

1. Calculate the Mean Absorbance of Calibrators 1-4 by referring to the table below.

Example of Calibrator 2 Calculation

Item	Absorbance
Calibrator 2: Well D1	0.469
Calibrator 2: Well E1	0.507
Total Absorbance of Calibrator 2	0.976
Mean Absorbance of Calibrator 2	0.488

2. Check the validation requirements below to determine if the test results are valid.

Item	Validation Requirements
Blank Well	Blank Absorbance should be < 0.050 if read at 450/630-700 nm Note: It should be < 0.100 if read at 450 nm
Calibrator 1	Mean Absorbance after subtraction of Blank Absorbance should be < 0.100
Calibrator 2	Mean Absorbance after subtraction of Blank Absorbance should be > 0.150
Calibrator 3	Mean Absorbance after subtraction of Blank Absorbance should be > 0.500
Calibrator 4	Mean Absorbance after subtraction of Blank Absorbance should be > 1.000

NOTE: The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

INTERPRETATION OF RESULTS

Qualitative

Calculate the Index Value to obtain qualitative specimen results.

1. If the test is valid, obtain Cut-Off Value by subtracting the Blank Absorbance from the Mean Absorbance of 1/2×(Calibrator 2+Calibrator 1). See an example of Cut-Off Value calculation below.

Item	Absorbance
Blank Absorbance: Well A1	0.011
Cut-Off Value: 1/2×(Mean Absorbance of Calibrator 2+ Mean Absorbance of Calibrator 1) – Blank Absorbance	1/2×(0.488+0.012)-0.011=0.239

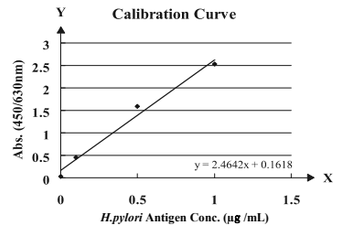
2. Calculate the Index Value by dividing the Specimen Absorbance by the Cut-Off Value, then read the results by referring to the Interpretation of Results table below.

Item	Absorbance
Specimen: Well F2	0.968
Blank Absorbance: Well A1	0.011
Cut-Off Value	0.239
Index Value: Specimen/Cut-Off Value	(0.968-0.011)/0.239=4.0

Quantitative

Draw the calibration curve and obtain quantitative specimen results.

1. Subtract the Blank Absorbance from the Mean Absorbance of each Calibrator, then plot them on the Y-axis against their concentration in µg/mL on the X-axis on a linear graph paper and draw the calibration curve. Draw the best fitted line through data points to obtain a standard curve. Refer to an example of the calibration curve at right.
   NOTE: Do not use the calibration curve at right to make any calculation. A calibration curve must be performed for each run.



2. Obtain quantitative specimen results from their absorbance by using the calibration curve.

NOTE: Specimens that have absorbance above Calibrator 4 should be pre-diluted using Extraction Solution and retested. The concentration must be multiplied by the dilution factor. Automated reading and calculation may also be performed using linear regression function on suitable computer programs.

Interpretation of Results - Qualitative and Quantitative

Results	Qualitative	Quantitative
	Index Value	Concentration
Negative	< 0.9	< 0.045 µg/mL
Positive	> 1.1	> 0.055 µg /mL
Equivocal*	≥ 0.9 and ≤ 1.1	0.045 – 0.055 µg/mL

***NOTE**: For Equivocal results, the specimen should be retested. Specimens that are repeatedly Equivocal after retest should be confirmed using an alternate method. If the results remain Equivocal, collect a new specimen in two weeks. If the new specimen is Positive, the specimen is presumed to be Positive.

LIMITATIONS

1. The *H. pylori* Antigen EIA Test Kit is used for the detection of *H. pylori* antigen in human stool. Diagnosis of an infectious disease should not be established based on a single test result. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A negative test result does not exclude the possibility of exposure. Specimens containing precipitate may give inconsistent test results.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
3. As with other sensitive immunoassays, there is the possibility that the positive result cannot be repeated due to inadequate washing from the initial test. The results may be affected due to procedural or instrument error.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The *H. pylori* Antigen EIA Test Kit has been compared to a leading commercial *H. pylori* Antigen EIA test using clinical specimens. The results show that the clinical sensitivity of the *H. pylori* Antigen EIA Test Kit is 98.6%, and the clinical specificity is 95.4%.

***H. pylori* Antigen EIA vs. Other EIA**

Method		Other EIA		Total Results
***H. pylori* Antigen EIA**	**Results**	Positive	Negative	
	Positive	70	6	76
	Negative	1	125	126
Total Results		71	131	202

Clinical Sensitivity: 98.6% (92.4-100.0%) * Clinical Specificity: 95.4% (90.3-98.3%)*
Overall Agreement: 96.5% (93.0-98.6%)* *95% Confidence Interval

Reproducibility

Intra-Assay: Within-run precision has been determined by using 10 replicates of two specimens: a low positive and a high positive.

Inter-Assay: Between-run precision has been determined by using 10 replicates on the same two specimens: a low positive and a high positive. Three different lots of the *H. pylori* Antigen EIA Test Kit have been tested using these specimens.

Specimen	Intra-Assay			Inter-Assay		
	Mean Absorbance/ Cut-Off	Standard Deviation	Coefficient of Variation (%)	Mean Absorbance/ Cut-Off	Standard Deviation	Coefficient of Variation (%)
1	1.741	0.156	8.96	1.723	0.133	7.72
2	4.726	0.252	5.33	4.861	0.252	5.18

BIBLIOGRAPHY

1. Marshall, BJ, McGechie, DB, Rogers, PAR and Glancy, RG. Pyloric *Campylobacter* infection and gastroduodenal disease. Med. J. Australia. (1985), 149: 439-44.
2. Soll, AH. *Pathogenesis of peptic ulcer and implications for therapy.* New England J. Med. *(1990), 322: 909-16.*
3. Ansorg, R, Von Recklinghausen, G, Pomarius, R and Schmid, EN. *Evaluation of techniques for isolation, subcultivation and preservation of Helicobacter pylori.* J. Clin. Micro. (1991), 29:51-53.
4. Pronovost, AP, Rose, SL, Pawlak, J, Robin, H and Schneider, R. *Evaluation of a new immunodiagnostic assay for Helicobacter pylori antibody detection: Correlation with histopathological and microbiological results*. J. Clin. Micro. (1994), 32: 46-50.
5. Megraud, F, Bassens-Rabbe, MP, Denis, F, Belbouri, A and Hoa, DQ. *Seroepidemiology of Campylobacter pylori infection in various populations*. J. Clin. Micro. (1989), 27: 1870-3.

Index of Symbols

	Consult instructions for use		Tests per kit		Manufacturer
IVD	For *in vitro* diagnostic use only		Use by	EC REP	Authorized Representative
2°C – 8°C	Store between 2-8°C	LOT	Lot Number	REF	Catalog #
H. pylori Antigen	*H. pylori* Antigen	Conjugate	Conjugate	Wash Buffer 25x	Wash Buffer (25x)
Extraction Solution	Extraction Solution	Substrate A	Substrate A	Substrate B	Substrate B
Stop Solution	Stop Solution	Calibrator 1	Calibrator 1	Calibrator 2	Calibrator 2
Calibrator 3	Calibrator 3	Calibrator 4	Calibrator 4	Package Insert	Package Insert
Microwell Plate	Microwell Plate	Plate Sealer	Plate Sealer		
Specimen Extraction Tube	Specimen Extraction Tube	Liquid Specimen Dropper	Liquid Specimen Dropper		

***ACON* Laboratories, Inc.**
10125 Mesa Rim Road,
San Diego, CA 92121, USA

EC REP
MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

Number: 1150475207
Effective date: 2015-07-31