# CoproELISA™ *H. pylori*

Enzyme-Linked Immunosorbent Assay (ELISA)
For the Detection of *Helicobacter pylori* antigens in human feces

## Instruction Manual

Test kit for 96 determinations
Catalog Number: 774-01

For *In Vitro* Diagnostic Use
Store at 2-8°C. **Do Not Freeze**

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**Intended Use**

Savyon's CoproELISA™ *H. pylori* is an Enzyme-Linked Immunosorbent Assay (ELISA) for use as a screening and detection test of *Helicobacter pylori* in human stool of suspected patient.

For ***In-Vitro*** Diagnostic Use

**Introduction**

*Helicobacter pylori (H. pylori)* is a Gram-negative, micro-aerophilic bacterium restricted to the stomach and the duodenum. It was identified in 1982 by Australian scientists Barry Marshall and Robin Warren, who found that it was present in patients with chronic gastritis and gastric ulcers, conditions that were not previously believed to have a microbial cause. *H. pylori* has also been associated as an etiologic agent in the development of duodenal ulcers and stomach cancer. More than fifty percent of the world's population harbor *H. pylori* in their upper gastrointestinal tract. However, over 80 percent of individuals infected with this bacterium are asymptomatic and it has been postulated that it may play an important role in the natural stomach ecology[1].

*H. pylori* is a contagious bacterium, although the exact route of transmission is not known[2,3]. Person-to-person transmission by either the oral-oral or fecal-oral route is most frequent. Consistent with these transmission routes, the bacteria have been isolated from feces, saliva and dental plaques of some infected patients. Transmission occurs mainly within families in developed nations, yet can also be acquired from the community in developing countries[4].

**Signs and symptoms:** Over 80% of people infected with *H. pylori* never experience symptoms or complications[5]. Acute infection may appear as an acute gastritis with abdominal pain (stomach ache) or nausea[6]. Where this develops into chronic gastritis, the symptoms, if present, are often those of non-ulcer dyspepsia: stomach pains, nausea bloating, belching and sometimes vomiting or black stool[6,7]

**Diagnosis:** Testing for *H. pylori* is recommended if peptic ulcer disease is suspected, low grade gastric MALT lymphoma, after endoscopic resection of early gastric cancer, if there are first degree relatives with gastric cancer, and unfrequently, in certain cases of dyspepsia. Laboratory diagnosis tests of *H. pylori* can be categorized as invasive (a biopsy check during endoscopy examined microscopically and also cultured), or noninvasive tests such as a blood antibody test, stool antigen test, or with the urea breath test (UBT) which involves the ingestion of isotopically labeled urea by the patient[8]. None of the test methods is completely failsafe. Even biopsy is dependent on the location of the biopsy and includes risk and discomfort to the patient and colonization in patches that might be missed by biopsy. The recent Maastricht 2-2000 Consensus Report recommends the use of the stool antigen and UBT tests as an aid in the diagnosis of *H. pylori* disease in the primary care setting[9].

CoproELISA™ *H. pylori* is a microwell based enzyme immunoassay for the detection of *Helicobacter pylori* antigens present in human feces.

**Principle of the test**

- Break-apart microwells are coated with monoclonal anti- *H. pylori* capture antibodies.
- Patient samples are diluted in H. pylori Stool Diluent and added to the microwells.
- A monoclonal antibody conjugated to horseradish peroxidase (HRP) is added to the microwells and incubated for 60 minutes at room-temperature.
- Unbound conjugate is removed by washing.
- TMB-substrate is added; the substrate is hydrolyzed by the peroxidase and yields a blue solution of the reduced substrate.
- Upon the addition of the stop solution, the blue color turns yellow and should be read by an ELISA reader at a wavelength of 450/620 nm.

**Summary of Manual Procedure**

Add 100 µl of Negative Control, Positive Control, and 100 µl of diluted specimens
↓
Add 50 µl of HRP-Conjugate (Ready to Use)
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Cover plate and incubate 60 min at room-temperature
↓
Wash 5 times with Wash Buffer (300 µl)

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Add 100 µl of TMB-Substrate

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Cover plate and incubate 15 min at room temperature

↓

Add 100 µl of Stop Solution

↓

Read absorbance at OD 450/620 nm

↓

Interpret results

## Kit contents for 96 determinations

1. **Microtiter plate coated with monoclonal anti- *H. pylori* capture antibodies:** 96 break-apart wells (8x12) coated with a monoclonal antibody specific for *H. pylori*, packed in an aluminum pouch containing a desiccant card.
   **1 plate**
2. **Concentrated Wash Buffer (20x):** PBS -Tween buffer
   **1 bottle, 100 ml**
3. **H. pylori Stool Diluent:** A ready-to-use buffer solution contains less than 0.05% Proclin as preservative. The Diluent is also to be used as the negative control solution (see TEST PROCEDURE)
   **2 bottles, 50 ml**
4. **HRP-Conjugate (green):** A ready-to-use solution containing Horseradish peroxidase (HRP) conjugated anti- *H. pylori* monoclonal antibody contains less than 0.05% Proclin as preservative.
   **1 bottle, 7 ml**
5. **Positive Control (red):** A ready to use solution containing *H. pylori* inactivated cells, contains less than 0.05% Proclin as preservative.
   **1 vial, 2.5 ml**
6. **TMB-Substrate:** A ready to use solution contains 3,3'5,5' tetramethylbenzidine as a chromogen and peroxide as a substrate.
   **1 bottle, 16 ml**
7. **Stop Solution:** A ready to use solution. Contains 1M $H_2SO_4$.
   **1 bottle, 16 ml**
8. **Disposable plastic pipettes:** **100 pc**
9. **Plate cover:** **1 unit**
10. **Instruction Manual** on website: www.sayvondiagnostics.com.downloads

## Materials Required But Not Supplied

1. Clean test tubes for dilution of patients' stools.
2. Adjustable micropipettes, or multichannel pipettes (50-200 and 200-1000µl ranges) and disposable tips.
3. Disposable plastic/wooden collectors or teaspoons.
4. One-liter volumetric flask.
5. One 50 ml volumetric cylinder.
6. Wash bottle.
7. Absorbent paper.
8. Vortex mixer.
9. ELISA-reader equipped with 450/620 nm filters.
10. Distilled or double de-ionized water.

## Warnings and Precautions

1. Reagents should be brought to room temperature before use.
2. When handling assay wells, avoid scratching the bottom of the wells because this may result in elevated absorbance readings.
3. Stool samples, microassay wells, micropipette tips and disposable stool collectors and tubes should be handled and disposed of as potentially biohazards after use. Wear gloves when doing the test.
4. **Unused microassay wells must be replaced in the re-sealable pouch with the desiccant to protect them from moisture.**
5. TMB-Substrate solution is an irritant material to skin and mucous membranes. Avoid direct contact.
6. Diluted sulfuric acid (1M $H_2SO_4$) is an irritant agent for the eyes and skin. In case of contact with eyes, immediately flush area with water and consult a physician).

## Storage and Shelf-Life of Reagents

1. The expiration date of the kit is given on the label. Expiration dates for each component are listed on individual labels. The kit should be stored between 2° and 8°C and should be returned to the refrigerator as soon as possible after use. Exposure of originally stoppered or sealed components to ambient temperature for a few hours will not cause damage to the reagents. **DO NOT FREEZE**!
2. Unused strips must be resealed in the aluminum pouch with the desiccant card, by rolling the open end and sealing tightly with tape over the entire length of the opening.

## Stool Collection

1. Standard collection and handling procedures used in-house for fecal specimens or culture are appropriate.
2. Preserved stool: **The test has not been confirmed with specimens after fixation**. (e.g. in 10% formalin, Sodium Acetate Formalin (SAF), or Polyvinyl Alcohol (PVA)).
3. Specimens should be kept between 2° and 8°C and tested within 48 hours after collection. If testing cannot be performed within 48 hours, store samples at -20°C, or lower.
4. Minimize specimen freezing and thawing which may cause degradation/proteolysis of the antigen and result in loss of activity.

## Test Procedure for manual use

### A. Preparation of Reagents

1. Bring all components and clinical specimens to be tested to room temperature. Determine the total number of specimens to be tested. In addition to the specimens, the following must be included in each test: one well of Negative Control (Use Stool Diluent for this purpose) and one well of Positive Control
2. Withdraw the microtiter plate from its aluminum pouch by cutting one end near the seal. Leave the required number

of strips (according to the number of specimens to be tested) in the 96 well frame.

3. Dilute the Concentrated Wash Buffer 1/20 with double-deionized or distilled water. For example, in order to prepare one liter of Wash Buffer, add 50 ml of the Concentrated Wash Buffer to 950 ml of double-deionized or distilled water.

**B. Sample Processing**

4. Set up one dilution tube for each specimen to be tested. 1.5 ml Eppendorf tubes are recommended for this purpose. Add 500 µl H. pylori Stool Diluent to each tube. Label the tube.
5. **Formed samples**: Use a wooden applicator stick or a disposable teaspoon to transfer the fecal specimen to the tube. Transfer approximately 0.1 to 0.15 g of specimen (about the size of a small pea) to the H. pylori Stool Diluent. Mix the collector in the Stool Diluent to remove as much sample as possible and squeeze the collector against the side of the tube to express any residual liquid.
   **Liquid samples**: transfer 100 µl of specimen to the tube. Make sure the liquid specimens are evenly suspended.
6. **Thoroughly mix (vortex) the fecal specimen for 15 sec. to ensure adequate sampling.** Let the tube stand for at least 10 minutes. Stool specimens may be centrifuged after dilution if required. Centrifuge the tubes at 1000 g for 30 sec. Ensure that the formed supernatant does not contain large particulate material.
7. Store the diluted samples between 2˚ to 8˚ C until test is performed.

**C. Incubation of stool samples and controls**

8. Pipette 100 µl of Positive control and Negative Control (i.e., H. pylori Stool Diluent) into separate wells of the test strip.
9. Dispense 100 µl of diluted stool samples into separate wells of the test strip using the provided disposable pipettes (the lowest mark on the pipette).
10. Dispense 50µl of ready-to-use conjugate into each well.
11. Cover the strips with a plate cover, gently shake the microplate for 20 sec to ensure homogenous mixing of the conjugate and sample (plate shaker can be used for this purpose). Incubate for 60 min at room-temperature.
12. **Washing step:** Discard the liquid content of the wells. Fill each well with Wash Buffer up to the end of the well (300µl). Repeat this step 4 times to a total of **FIVE times**. Automatic washing machine can be used.
13. Dry the strips and frame by gently tapping them over clean absorbent paper.

**D. Incubation with TMB Substrate**

14. Dispense 100 µl of TMB-Substrate into each well, cover the strips with a plate cover, and incubate at room temperature for **15 minutes**.
15. Stop the reaction by adding 100µl of Stop Solution (1M $H_2SO_4$) into each well.

**E. Determination of Results**

16. Determine the absorbance at 450/620 nm and record the results. Determination should not exceed 10 minutes following stopping of the chromogenic reaction.

**Note:** Any air bubbles should be removed before reading. The bottom of the ELISA plate should be carefully wiped.

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**Test Procedure for automation use**

**A. Preparation of Reagents**

1. Bring all components and clinical specimens to be tested to room temperature. Determine the total number of specimens to be tested. In addition to the specimens, the following must be included in each test: one well of Negative Control (Use H.pylori Stool Diluent for this purpose) and one well of Positive Control
2. Withdraw the microtiter plate from its aluminum pouch by cutting one end near the seal. Leave the required number of strips (according to the number of specimens to be tested) in the 96 well frame.
3. Dilute the Concentrated Wash Buffer 1/20 with double-deionized or distilled water. For example, in order to prepare one liter of Wash Buffer, add 50 ml of the Concentrated Wash Buffer to 950 ml of double-deionized or distilled water.

**B. Sample Processing**

4. Set up one sample's dilution tube for each specimen to be tested (use sample's tubes compatible with the available automation equipment). Add 1000 µL H. pylori Stool Diluent to each sample's tube. Label the tube.
5. **Formed samples**: Use a wooden collector or a disposable teaspoon to add the fecal specimen to the sample's tube. Transfer approximately 0.2 to 0.3 g of specimen (about the size of 2 small peas) to the sample's tube. Mix the collector in the H. pylori Stool Diluent to remove as much sample as possible and squeeze the collector against the side of the tube to extract any residual liquid.
   **Liquid samples**: transfer 300 µL of specimen to the tube. Make sure the liquid specimens are evenly suspended.
6. **Thoroughly mix (vortex) the fecal specimen to ensure adequate sampling.**
7. Let the tube stand for at least 10 minutes until large particulate matter is precipitated (decantation). Ensure that the formed supernatant does not contain large particulate material. In case and required centrifuge the tubes at 1000 g for 30 sec.
8. Transfer the sample's tubes to the corresponding rack at the automation machine.

**C. Incubation of stool samples and controls with conjugate**

9. Pipette 100µl of Positive control and 100µl of Negative Control (i.e., H. pylori Stool Diluent) into separate wells of the test strip.

10. Dispense 100µl of diluted stool samples into the test strip. Each sample in a different well.
11. Dispense 50µl of ready-to-use conjugate into each well, gently shake the microplate for 10 sec.
12. Incubate the plate for **60 minutes** at room temperature.
13. Perform 5 X 350µl wash cycles using the pre-diluted Wash Buffer.
14. Perform 2 aspirate cycles with aspirate sweep.

**D. Incubation with TMB Substrate**

15. Dispense 100µl of TMB-Substrate into each well. Incubate at room temperature for 15 minutes.
16. Stop the reaction by adding 100µl of Stop Solution (1M $H_2SO_4$) into each well.

**E. Determination of Results**

17. Determine the absorbance at 450/620 nm and record the results.

**Please note that each automation machine has specific technical commands. Please implement Savyon's automation procedure for this kit on the operation protocol of your automation equipment.**

## Test Validation

For the test to be valid, the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

**Negative Control (NC):**

The absorbance value should be < 0.15 at 450/620 nm.

**Positive Control (PC):**

The absorbance value should be ≥ 0.8 at 450/620 nm.

## Interpretation of Results

Spectrophotometric Dual Wavelength at 450/620 nm

**Negative**: $OD_{sample} < 0.150$
**Positive**: $OD_{sample} \geq 0.150$

## Test Limitations

1. CoproELISA™ *H. pylori* detects *H. pylori* antigen in fecal specimen. Antigen detection in stools should be taken into account by physician in light of the patient's clinical history and results before making a final diagnosis. Inability to detect *H. Pylori* antigens in patient's fecal samples may not preclude actual disease but may be caused by other factors such as incorrect sampling handling or storage of stools. It is also possible that *H. Pylori* levels are below the kit's limit detection.
2. The test is qualitative and no quantitative interpretation should be made with respect to the values.
3. The stability of *H. pylori* in stool samples may be affected. Therefore, it is important to keep samples at 2-8˚ C soon after collection. Samples that are not analyzed within 48 hours may be frozen and thawed.
4. Some samples may give low detection levels. This could be caused by a number of reasons such as the presence of bacteria at low levels, or by factors in the feces that interfere with immuno- detection of the test. Under these conditions it is recommended to retest samples using fresh specimen.

## Performance Characteristics of the Test

Clinical evaluations performed with the CoproELISA™ *H. pylori* test demonstrated that an ELISA-based assay could reliably and predictably detect *H. pylori* antigen in human stool in symptomatic patients.

Clinical stool specimens were evaluated by CoproELISA™ *H. pylori* test on a total of 145 samples and were compared to results of an FDA-approved commercial reference ELISA kit.

**Table 1:**

| | | ELISA Reference Kit* | | |
|---|---|---|---|---|
| | | **positive** | **negative** | **total** |
| **CoproELISA™ *H. pylori*** | **positive** | 91 | 2 | 93 |
| | **negative** | 4 | 48 | 52 |
| | **total** | 95 | 50 | 145 |

*** FDA / CE approved ELISA kit**

**Sensitivity: 96 %, Specificity: 96** %
PPV: 98%, NPV: 92%

## Cross Reactivity and Interference by Mixed infections

The CoproELISA™ *H. pylori* test was evaluated using clinical stool specimens defined as positive for various gastrointestinal pathogens. No cross-reactivity of interference by mixed infection with any of the gastrointestinal pathogens listed below:

*Salmonella spp. Campylobacter, Shigella, Dientamoeba fragilis, Blastocystis, Giardia lamblia, Entamoeba dispar, Entamoeba histolytica and Clostridium difficile*

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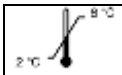
|  | Temperature Limitation |
|  | Consult instructions for use |
| IVD | In Vitro Diagnostic Medical Device |
|  | Manufacturer |
| EC REP | Authorized European Representative |