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EDI™ Fecal H. pylori Antigen ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Qualitative Detection of Helicobacter pylori Antigen in Feces

REF **KT 824** EU: IVD CE Σ 96 +2 +8 °C

I. INTENDED USE

This microplate-based ELISA (enzyme linked immunosorbent assay) kit is intended for the qualitative detection of *Helicobacter pylori* antigen in feces. The assay is a useful tool in the diagnosis of active *H. pylori* infection.

II. SUMMARY OF PHYSIOLOGY

H. pylori (previously known as *Campylobacter pyloridis*) is a type of bacterium that infects the stomach and is a common cause of peptic ulcers. *H. pylori* bacteria can be passed from person to person through direct contact with saliva, vomit or fecal matter. *H. pylori* can also be spread through contaminated food or water. The infection is normally acquired during childhood. *H. pylori* usually goes undiagnosed until symptoms of a peptic ulcer occur. *H. pylori* infection is quite common and is present in about half the people in the world.[1]

III. ASSAY PRINCIPLE

This "sandwich" ELISA is designed, developed and produced for the qualitative measurement of *H. pylori* antigen in stool specimen. The assay utilizes the microplate-based enzyme immunoassay technique by coating highly purified anti-*H. Pylori* antibody onto the wall of microtiter wells. Assay controls and extracted fecal specimen are added to microtiter wells of microplate that was coated with a highly purified monoclonal *H. pylori* antibody on its surface. During the assay, the *H. pylori* antigen will be bound to the antibody coated plate after an incubation period. The unbound material is washed away and another HRP-conjugated monoclonal antibody, which specifically recognizes the protein of *H. pylori* is added for further immunoreactions. After an incubation period, the immunocomplex of "*H. pylori* Antibody – *H. pylori* Antigen – HRP-conjugated Anti-*H. pylori* Tracer Antibody" is formed, if *H. pylori* antigen is present in the test sample. The unbound tracer antibody and other proteins in buffer matrix are removed in the subsequent washing step. HRP conjugated tracer antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to *H. pylori* proteins captured on the wall of each microtiter well is directly proportional to the amount of *H. pylori* antigen level in each test specimen.

IV. REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8 °C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use, allow all reagents to equalize to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

1. H. pylori Antibody Coated Microplate (Cat. No. 30665)
One microplate with 12 x 8 strips (96 wells total) coated with highly purified H. pylori antibody. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

2. Anti-H. pylori Tracer Antibody (Cat. No. 30666)
One vial containing 12 mL ready-to-use horseradish peroxidase (HRP)-conjugated monoclonal *H. pylori* antibody in a stabilized protein matrix. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

3. ELISA HRP Substrate (Cat. No. 10020)
One bottle containing 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

4. ELISA Stop Solution (Cat. No. 10030)
One bottle containing 12 mL of 0.5 M sulfuric acid. This reagent should be stored at 2 – 8 °C or room temperature and is stable until the expiration date on the kit box.

5. H. pylori Positive Control (Cat. No. 30810)
One vial contains 1 mL of positive control (30810). This control is in a liquid bovine serum albumin-based matrix with mercury and sodium azide preservative. This reagent should be stored at 2 – 8 °C, -20ºC for long term storage.

6. ELISA Wash Concentrate (Cat. No. 10010)
One bottle containing 30 mL of 30-fold concentrate. Before use the contents must be diluted with 870 mL of distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide and non-mercury based preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiration date on the kit box.

7. H. Pylori Concentrated Assay Buffer (Cat. No. 30669)
One bottle containing **30 mL** of **4-fold** concentrated buffer matrix with protein stabilizers and preservative. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box. Before use the concentrated buffer must be diluted with **90 mL** of demineralized water and mixed well. Upon dilution, this yields as a **negative control** and **patient sample diluent** containing a surfactant in phosphate-buffered saline with a non-azide preservative. The diluted reagent is stored at 2 – 8 °C this reagent is stable until the expiration date on the kit box.

V. SAFETY PRECAUTIONS

The reagents must be used in a laboratory and are for professional use only. Materials sourced for reagents containing bovine serum albumin were derived in the contiguous 48 United States and obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

- Precision single channel pipettes capable of delivering 10 µL, 50 µL, 100 µL, and 1000 µL, etc.
- Repeating dispenser suitable for delivering 100 µL.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
- Disposable plastic 1000 mL bottle with cap.
- Aluminum foil.
- Deionized or distilled water.
- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

VII. SPECIMEN COLLECTION & STORAGE

Fresh fecal sample should be collected into a stool sample collection container. It is required to collect a minimum of 1-2 mL liquid stool sample or 1-2g solid sample. The collected fecal sample must be transported to the lab in a frozen condition (-20ºC). If the stool sample is collected and tested the same day, it is allowed to be stored at 2-8ºC.

VIII. ASSAY PROCEDURE

1. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
(2) Concentrated Assay Buffer (Cat. 30669) must be diluted to working solution prior use. Please see REAGENTS section for details.
(3) ELISA Wash Concentrate (Cat. 10010) must be diluted to working solution prior use. Please see REAGENTS section for details.

2. Patient Sample Preparation

2.1 For manual weighing procedure only:

Patient samples need to be diluted **1:24** with 1x Assay Buffer before being measured.

(1) Label a test tube (12x75 mm) or a 4 ml plastic vial.
(2) With solid stool sample, take or weigh an equivalent amount (about **40mg,** size as a grain of rice) with a spatula or a disposable inoculation loop. Suspend the solid stool sample with **1 mL 1x Assay Buffer** and mix well on a vortex mixer.
(3) Centrifuge the diluted fecal sample at 3000 rpm (800-1500 g) for 5-10 minutes. The supernatant can be directly used in the assay. As an alternative to centrifuging, let the diluted samples sit and sediment for 30 minutes and take the clear supernatant for testing.
Note: If the test procedure is performed on an automated ELISA system, the supernatant must be particle-free by centrifuging the sample**.**
(4) This sample can be stored at 2-8ºC up to three (3) days and below -20ºC for longer storage. Avoid more than 3x freeze and thaw cycle.

2.2 Using EDI Fecal Sample Collection Device, (Cat. KT854)

(1) Label a Fecal Sample Collection tube
(2) Continue assay by following the instructions on the Sample Collection Tube insert, KT854.
(3) Centrifuge the diluted fecal sample at 3000rpm (800 - 1500 g) for 5-10 minutes. As an alternative to centrifuging, let the diluted samples sit and sediment for 30 minutes and take the clear supernatant for testing.
Note: If the test procedure is performed on an automated ELISA system, the supernatant must be particle-free by centrifuging the sample**.**
(4) This sample can be stored at 2-8ºC up to three (3) days and below -20ºC for longer storage. Avoid more than 3x freeze and thaw cycle.

3. Assay Procedure

(1) Place a sufficient number of anti-*H. Pylori* antibody coated microwell strips (Cat. 30665) in a frame to run *H. Pylori* negative control (1x Assay buffer), positive control (Cat#30810) and unknown samples in duplicate.
(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	Control Negative	SAMPLE 3	SAMPLE 7
B	Control Negative	SAMPLE 3	SAMPLE 7
C	Control Positive	SAMPLE 4	SAMPLE 8
D	Control Positive	SAMPLE 4	SAMPLE 8
E	SAMPLE 1	SAMPLE 5	SAMPLE 9
F	SAMPLE 1	SAMPLE 5	SAMPLE 9
G	SAMPLE 2	SAMPLE 6	SAMPLE 10
H	SAMPLE 2	SAMPLE 6	SAMPLE 10

(3) Add **100 µL** of controls (use 1x Assay buffer as a negative control) and diluted patient stool samples into each designated microwell. Mix gently by tapping the plate.
(4) Cover the plate with a plate sealer and also with aluminum foil to avoid exposure to light.
(5) Incubate plate at room temperature for **1 hour**
(6) Remove plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used
(7) Add **100 µL** of Anti-H.pylori tracer antibody solution (30666) to each of the wells. Mix by gently tapping the plate.
(8) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
(9) Incubate plate at room temperature for **30 minutes**.
(10) Remove plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used
(11) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
(12) Cover the plate with aluminum foil to avoid exposure to light.
(13) Incubate plate at room temperature for **20 minutes**

(14) Remove the aluminum foil. Add **100 µL** of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
(15) Read the absorbance at 450 nm.

IX. PROCEDURAL NOTES

1. It is recommended that all controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.

X. INTERPRETATION OF RESULTS

Visual:

1. Positive or reactive: Any sample well that is obviously more yellow than the negative control well.
2. Negative or non-reactive: Any sample well that is not obviously more yellow than the negative control well.

Note: The negative control, as well as some patient samples, may show some slight yellow color. A sample well must be obviously darker or more yellow than the negative control well, when it is interpreted as a positive result.

ELISA Reader:

1. Calculate the average absorbance for each pair of duplicate test results.
2. Calculate the cut-off
   The positive cut-off and the negative cut-off are established by using following formula.

   Positive Cut-Off = 1.1 x (mean extinction of negative control + 0.10)
   Negative Cut-Off = 0.9 x (mean extinction of negative control + 0.10)

3. **Interpret test result**
   - Positive: patient sample extinction is greater than the Positive Cut-Off
   - Negative: patient sample extinction is less than the Negative Cut-Off
   - Equivocal: patient sample extinction is between the Positive Cut-Off and the Negative Cut-Off.

4. **Assay quality control**
   - Positive control must show an average OD reading greater than 0.8.
   - Negative control should show an average OD reading less than 0.09.

XI. EXAMPLE DATA AND CALCULATED CUT-OFF

A typical absorbance data and the resulting negative control and positive controls are represented. This absorbance must not be used in lieu of control values run with each assay.

	OD 450 nm	Average OD 450 nm
Negative Control	0.049 0.050	0.050
Positive Control	1.332 1.376	1.354

Positive Cut-Off = 1.1 x (0.050 + 0.10) = 0.165
Negative Cut-Off = 0.9 x (0.050 + 0.10) = 0.135

XII. EXPECTED VALUES

Stool samples from 29 negative specimens and 17 positive specimens were tested with this ELISA.

Epitope's ELISA \ Samples	True Positive	True Negative	Total
Positive	17	0	17
Negative	0	29	29
Total	17	29	46

Sensitivity: 100% (17/17)
Specificity: 100% (29/29)
Accuracy: 100% (46/46)

XIII. LIMITATION OF THE PROCEDURE

(1) The results obtained with this Fecal *H. pylori* antigen test kit serve only as a useful aid to diagnosis. However, the test results should not be interpreted as diagnostic in themselves.
(2) Bacterial or fungal contamination of stool specimens or reagents, or cross-contamination between reagents may cause erroneous results.
(3) Water deionized with polyester resins may deactivate the horseradish peroxidase enzyme.

XIV. QUALITY CONTROL

To assure the validity of the results each assay must include both negative and positive controls. For a valid test, the positive control must have an absorbance of at least 0.8 OD units and the negative control must be less than 0.09 OD units. We also recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

To order EDI H. pylori controls. Please order H. pylori control 1 (Cat# 30825), Control 2 (Cat# 30826), or Control set (Cat#30827.)

XV. PERFORMANCE CHARACTERISTICS

Reproducibility
The reproducibility of this assay is validated by measuring four samples (two negative and two positive) both in a single assay of 12-replicate determinations and in 6 different assays run on different dates. The results showed a consistent test results interpretation for all the samples.

Specificity
The assay does not cross react to following organisms: Cryptosoridium parvum, Giardia.

XVI. WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state

XVII. REFERENCES

1: Ungar BL, Yolken RH, Nash TE, Quinn TC. Enzyme-linked immunosorbent assay for the detection of H. Pylori in fecal specimens. J Infect Dis. 1984 Jan;149(1):90-7.
2: Rosenblatt JE, Sloan LM, Schneider SK. Evaluation of an enzyme-linked immunosorbent assay for the detection of H. Pylori in stool specimens. Diagn Microbiol Infect Dis. 1993 May-Jun;16(4):337-41.
3: Stibbs HH, Samadpour M, Manning JF. Enzyme immunoassay for detection of H. Pylori cyst antigens in formalin-fixed and unfixed human stool. J Clin Microbiol. 1988 Sep;26(9):1665-9.
5: Stibbs HH. Monoclonal antibody-based enzyme immunoassay for H. Pylori antigen in human stool. J Clin Microbiol. 1989 Nov;27(11):2582-8.
8: Janoff EN, Smith PD, Blaser MJ. Acute antibody responses to H. Pylori are depressed in patients with AIDS. J Infect Dis. 1988 Apr;157(4):798-804.

This product was developed and is manufactured by
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Manufacturer	No. of tests
REF Catalog Number	Keep away from heat and direct sun light
CONC Concentrate	Store at
Read instructions before use	Use by
IVD In Vitro Diagnostic Device	LOT Lot No.
EC REP Authorized Representative In Europe	

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