EPITOPE DIAGNOSTICS, INC.

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 CE IVD

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. This kit is for in vitro diagnostic use only.

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.

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.

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.

1. H.pylori Antibody Coated Microplate (30665)

Microplate coated with H.pylori antibody.
Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to Use

2. Anti-H.pylori Tracer Antibody (30666)

HRP-conjugated monoclonal H.pylori antibody in a stabilized protein matrix.
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use

3. ELISA HRP Substrate (10020)

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use

4. ELISA Stop Solution (10030)

0.5 M sulfuric acid
Qty: 1 x 12 mL
Storage: 2 – 25°C
Preparation: Ready to Use

5. H.pylori Positive Control (30810)

Liquid bovine serum albumin-based matrix with sodium azide preservative.
Qty: 1 x vial
Storage: 2 – 8°C
Preparation: Ready to Use

6. ELISA Wash Concentrate (10010)

Surfactant in a phosphate buffered saline with non-azide preservative.
Qty: 1 x 30 mL
Storage: 2 – 25°C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

7. H.pylori Concentrated Assay Buffer (30669)

Concentrated buffer matrix with protein stabilizers and a non-azide preservative. Upon dilution, this yields a negative control and a patient sample diluent.
Qty: 1 x 30 mL
Storage: 2 – 8°C
Preparation: 4X Concentrate. The contents must be diluted with 90 mL distilled water and mixed well before use.

SAFETY PRECAUTIONS

The reagents are for in vitro diagnostic use only. Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was 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 were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. 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.

MATERIALS REQUIRED BUT NOT PROVIDED

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

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.

ASSAY PROCEDURE

1. Reagent Preparation

1. Prior to use allow all reagents to come to room temperature (20-25 ºC). Reagents from different kit lot numbers should not be combined or interchanged.
2. Concentrated Assay Buffer (30669) must be diluted to working solution prior use. Please see REAGENTS section for details.
3. ELISA Wash Concentrate (10010) must be diluted to working solution prior use. Please see REAGENTS section for details.

2. Specimen Preparation

2.1 Manual Weighing Procedure

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** 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.
4. Note: If the test procedure is performed on an automated ELISA system, the supernatant must be particle-free by centrifuging the sample**.**
5. (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 (KT-854)

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.
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.

*Note: If the test procedure is performed on an automated ELISA system, the supernatant must be particle-free by centrifuging the sample**.***

3. Assay Procedure

1. Place a sufficient number of microwell strips (30665) in a holder to run controls (Negative Control (diluted Assay Buffer (30669), Positive Control (30810)), and diluted samples in duplicate.
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Negative Control	SAMPLE 3	SAMPLE 7
B	Negative Control	SAMPLE 3	SAMPLE 7
C	Positive Control	SAMPLE 4	SAMPLE 8
D	Positive Control	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 (Negative Control (diluted Assay Buffer (30669), Positive Control (30810)), and diluted samples into the designated microwells. Mix by gently tapping the plate.
4. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **60 minutes.**
5. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
6. Add **100 µL** of Anti-H.pylori Tracer Antibody (30666) to all wells. Mix by gently tapping the plate.
7. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **30 minutes.**
8. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
9. Add **100 µL** of ELISA HRP Substrate (10020) into each of the wells. Mix by gently tapping the plate.
10. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
11. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (10030) into each of the wells. Mix by gently tapping the plate.
12. Read the absorbance at **450nm** within **10 minutes** with a microplate reader.

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.

INTERPRETION OF RESULTS

1. 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.

2. ELISA Reader:

1. Calculate the average absorbance for each pair of duplicate test results.
2. Calculate the cut-off.
   - 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 the rest 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.
   - Blank well should show an average OD reading less than 0.18.

LIMITATIONS 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.

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 0.18 OD units. We also recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

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

EXPECTED VALUES

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

Samples / Epitope's ELISA	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)

EXAMPLE DATA

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.

Note: This curve should not be used in lieu of calibrator curve run with each assay.

Well ID	Reading Absorbance (450 nm)	
	Readings	Average
Negative Control	0.049	0.050
	0.050	
Positive Control	1.332	1.354
	1.376	

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

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

PERFORMANCE CHARACTERISTICS

Specificity

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

Reproducibility and Precision

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.

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.

REFERENCES

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

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Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

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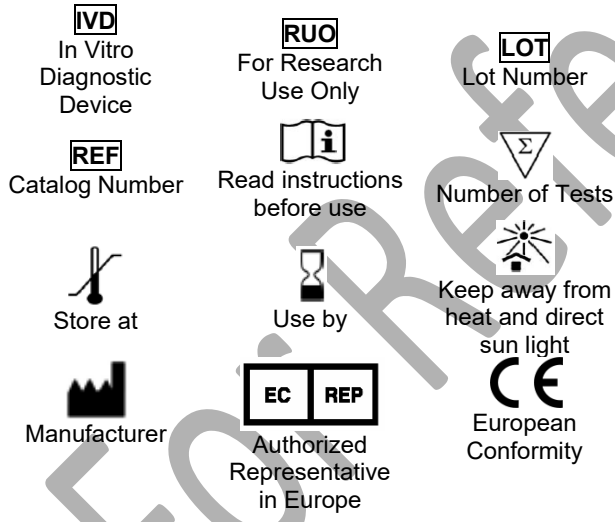
GLOSSARY OF SYMBOLS (EN 980/ISO 15223)



SHORT ASSAY PROCEDURE

1. Add **100 µL** of the blank, controls, and diluted samples into the designated microwells.
2. Mix, cover, and incubate at **room temperature (20-25 ºC)** with for **60 minutes**.
3. Wash each well five times.
4. Add **100 µL** of the tracer antibody to each well.
5. Cover and incubate at **room temperature (20-25 ºC)** for **30 minutes**.
6. Wash each well five times
7. Add **100 µL** of substrate to each well.
8. Cover and incubate at **room temperature (20-25 ºC)** for **20 minutes**.
9. Add **100 µL** of the stop solution to each well.
10. Read the absorbance at **450 nm.**