EPITOPE DIAGNOSTICS, INC.

EDI™FecalHelicobacter pylori Antigen CLIA Kit

Chemiluminescence Immunoassay (CLIA) for the Quantitative Measurement of Helicobacter pylori Antigenin Feces.

REF CL0826 CE IVD 100,150,250 +2 +8 °C

INTENDED USE

This Chemiluminescence Immunoassay (CLIA) kit is intended for the quantitative determination of Helicobacter pylori (*H. pylori*) antigen levels in feces using the ECL100 or ECL25 Immunoassay analyzer. It is for in-vitro diagnostics use only.

SUMMARY OF PHYSIOLOGY

H. pylori (previously known as *Campylobacter pyloridis*) is a type of bacteria that infects the stomach and is a common cause of chronic gastritis and 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 CLIA is designed, developed, and produced for the quantitative measurement of *H. pylori* antigeninfecal samples. The assay utilizes a two-site “sandwich” technique with two antibodies that bind to different epitopes of *H. pylori*antigen.

Assay calibrators, controls, or extracted patient fecal samples are added directly to a reaction vessel. Simultaneously, a biotinylated antibody, a streptavidin coated magnetic particle and subsequentlyanacridinium ester conjugated antibodyare added to the vessel. The magnetic particles capture the biotin antibody as well as an immuno complex in the form of “magnetic particles – biotinylatedanti-H. pylori antibody –H. pylori antigen– acridinium ester conjugated anti-H. pylori antigen antibody”.

The materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the trigger solution is added to the reaction vessel and light generated by the reaction is measured with the ECL-100 or ECL-25analyzer. The relative light units (RLU) are proportional to the concentration of H. pylori antigen in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in fecal H. pylori antigen concentration.

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.Reagents from different kit lot numbers should not be combined or interchanged.

1. H. pylori Magnetic Particle Solution (L0521)

Qty:	1 x 2.3 mL (100/kit), 2x 2.0 mL (150/kit), 2 x 2.7 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to Use

2. Biotin H. pylori Antibody (L0522)

Qty:	1 x 8.5 mL (100/kit), 1 x 13 mL (150/kit), 1 x 20 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to Use

3. Acridinium Ester H. pylori Antibody (L0523)

Qty:	1 x 8.5 mL (100/kit), 1 x 13 mL (150/kit), 1 x 20 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to Use

4. Fecal H. pylori Antigen Calibrators(L0526-L0527)

Liquid H. pylori in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.

Qty:	2 x vials
Storage:	2 – 8°C before reconstitution, <-20°C after reconstitution; Do not exceed 6 freeze-thaw cycles.
Preparation:	1.0 mL of Calibrators, mix by inversions or gentle vortexing. Make sure that Calibrators is well mixed before use.

5. Fecal H. pylori Antigen Controls (L0528-L0529)

Liquid H. pylori in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.

Qty:	2 x vials
Storage:	2 – 8°C before reconstitution, <-20°C after reconstitution; Do not exceed 6 freeze-thaw cycles.
Preparation:	1.0 mL of control, mix by inversions or gentle vortexing. Make sure that controlis well mixed before use.

6. Concentrated Fecal Extraction Buffer (30669)

Concentrated buffer matrix with protein stabilizers and preservativewhich serves as a patient sample diluent containing a surfactant in phosphate-buffered saline with a non-azide preservative.

Qty:	1 x 60 mL
Storage:	2 – 8°C
Preparation:	4X Concentrate. The contents must be diluted with 180 mL distilled water and mixed well before use.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for in vitro diagnostic use. 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. 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

The instrument only uses materials supplied by Epitope Diagnostics, Inc. When materials available from third-party suppliers are used, Epitope Diagnostics, Inc. takes no responsibility for the validity of results obtained. Material is available for purchase from Epitope Diagnostics, Inc. Please contact your distributor for more information.

1. ECL100 Immunoassay Analyzer or ECL25 Immunoassay Analyzer
2. CL011 Cuvettes (for ECL100) or CL010 Cuvettes (for ECL25)
3. Wash Reagent (P-594)
4. Trigger Solutions A and B (P-595)

SPECIMEN COLLECTION AND PREPARATION

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- 5 grams 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 in the same day, it is allowed to be stored at 2-8ºC.

Patient samples need to be diluted **1:11**with patient sample diluent before being measured. A 1x working solution of concentrated fecal sample extraction buffer (30669)is suggested in the extraction of samples used with this assay.

1. Label a test tube (12x75mm) or a 2.5 mL plastic vial.
2. Add 1 mL of diluted fecal sample extraction buffer to each tube or vial.
3. Add 100 µL of liquid stool sample to the above tube.
4. With solid stool sample, take an equivalent amount (about 80-120 mg) with a spatula or a disposable inoculation loop. Vigorously mix or vortex to dissolve stool specimen in the tube.
5. Let the extracted samples sit and sediment for 15 minutes. Make sure there is not free particle on the surface of liquid supernatant. Load the tube for sample test. Alternatively, centrifuge the extracted fecal sample at 1000 rpm (200 g) for 3 minutes before loading the tube for testing.

Note: The supernatant MUST be particle free to avoid damaging the ECL100 or ECL25 instrument. If necessary, remove the supernatant into an empty tube to ensure that no particles are present.

CALIBRATION

An active calibration curve is required for all tests. For the assay, calibration is required for the first time use of a reagent lot and every 14 days thereafter or when either kit control is out of range. Refer to appropriate system manuals for configuring calibrators.

QUALITY CONTROL

The characteristics of patient samples are simulated through controls and are critical to validate the performance of CLIA assays due to the random access format. Use of controls is left to the discretion of the user, based on good laboratory practices, requirements, and applicable laws. We suggest performing a control test once every day. Quality control results that do not fall within acceptable ranges may indicate invalid test results.

ASSAY PROCEDURE

1. Reagents from different kit lot numbers should not be combined or interchanged. Make sure that there are no air bubbles in any reagents, calibrator and control vials.

2. Reagent Preparation

2.1 Remove reagent cartridges from packaging and replace the solid caps with the provided soft caps for ECL100. For ECL25, carefully remove the aluminum foil seal on each container on the cartridges and insert soft caps.

2.2 For the ECL100, take out the Magnetic Particle bottle make sure to roll between hands and gently but thoroughly mix until the magnetic particle solution is homogenous. The solution should be uniform with no clumps of magnetic particles visible; this step is vital for assay performance. For ECL25, mix the magnetic beads by moving back and forth the bottom part of the cartridge at upright position. Make sure to look inside the cartridge until the solution is uniform with no clumps of magnetic particles visible and no air bubbles. Recap the bottle. Open the top soft cap of all reagent bottles, leaving only the hollow soft rubber. The reagents are now ready to be loaded into the ECL100 or ECL 25 for calibration.

3. Assay Program

The following table illustrates the protocol used by the ECL100 or ECL25 for instrument operation.

Component	Quality Control Hole (µL)	Sample Hole (µL)
H. pylori Antigen Controls (L0528-L0529)	50	-
Extracted Fecal Samples	-	50
Biotin H pylori Antibody (L0522)	75	75
H. pylori Magnetic Particle Solution (L0521)	20	20
Incubation Period 1		
Wash the reaction cup 3 times with the wash reagent.		
Acridinium Ester H. pylori Antibody (L0523)	75	75
Incubation Period 2		
Wash the reaction cuvette 3 times with wash reagent.		
Trigger Solution A (P-595)	100-200	100-200
Trigger Solution B (P-595)	100-200	100-200

The assay total incubation time is less than 30 minutes.

INTERPRETATION OF RESULTS

The chemiluminescence analyzer calculates the concentration values of the sample and the control by a standard curve (fitting method: four parameters or point-to-point) and the measured RLU. Values are compared with the range of the marked value. If it exceeds the indicated quality control range, it indicates that the test is unqualified and needs to be re-tested.

Due to methodological differences or antibody specificity, there may be deviations between the test results of reagents from different manufacturers.Therefore, direct comparisons should not be made to avoid false interpretation.

EXPECTED VALUES

Fecal H. pylori antigen concentrations were measured in stool samples collected from 45 apparently healthy adults using the EDI™ Fecal H. pylori Antigen CLIA Kit. The suggested normal cut off is 1.5 ng/mL.

It is highly recommended that each laboratory should establish their own normal range for fecal H. pylori antigen based on local populations.

LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 or ECL25 Immunoanalyzer only. Refer to the appropriate system manuals for a specific description of installation, start-up, operation, system performance, instructions, calibration, precautions, hazards, maintenance, and troubleshooting.
2. Reagents from different lots cannot be mixed.
3. Test results from this product should not be the sole basis for clinical diagnosis.
4. If the test sample result is higher than the upper limit of the calibration curve, it is recommended to re-measure after

dilution according to a certain ratio. The measurement result is recalculated according to the dilution ratio to ensure the accuracy of the result.

PERFORMANCE CHARACTERISTICS

Hook Effect

The assay shows no hook effect up to 2,000 ng/mL.

Limit of Blank

The limit of blank (LoB) was determined by 20 replicates of calibrator matrix to be 0.037 ng/mL.

Limit of Detection

The limit of detection (LoD) was determined by 20 replicates of low-level samples to be 0.112 ng/mL.

Limit of Quantification

The limit of quantification (LoQ) was determined by 20 replicates of low-level samples to be 0.262 ng/mL.

Linearity

Linearity was determined by measuringtwo diluted standards (L6 and L5) of high H. pylori antigen concentration. In each assay, the average of three replicates of each of the diluted standards is used for a correlation analysis against calculated theoretical values.

Standard L6	Average Measured Concentration (ng/mL)	Theoretical Concentration (ng/mL)	CV (%)	R^2
10%	10.6	12.5	4.2	0.996
20%	20.4	24.9	2.8	
40%	42.9	49.8	3.8	
60%	63.9	74.7	0.5	
80%	93.4	99.6	1.7	
100%	124.6	124.6	3.9	
Standard L5	**Average Measured Concentration (ng/mL)**	**Theoretical Concentration (ng/mL)**	**CV (%)**	**R2**
10%	4.7	4.7	7.1	0.998
20%	7.8	9.4	2.4	
40%	16.1	18.8	0.7	
60%	26.2	28.2	0.5	
80%	34.3	37.6	3.9	
100%	45.3	47.0	1.2	

Repeatability

Reproducibility was determined by measuring ten replicates of two control samples.

Control	Average Concentration (ng/mL)	CV (%)
1	9.9	5.5
2	29.1	1.9

Accuracy

Accuracy was determined by three replicates of twostandards used to generate the multi-point calibration curve.

Standards	Measured Concentration (ng/mL)	Average Concentration (ng/mL)	Target Value ± 15% (ng/mL)
L3	5.9	5.6	4.6 – 6.2
	6.0		
	4.9		
L5	51.2	50.1	41.4 – 56.0
	50.7		
	48.4		

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.

This product is manufactured by
EpitopeDiagnostics, Inc.
7110 Carroll Road
San Diego, CA 92121, USA

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

EC	REP

MDSS GmbH
Schiffgraben 41,
30175 Hannover, Germany

GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

IVD — In Vitro Diagnostic Device

CE — European Conformity

LOT — Lot Number

REF — Catalog Number

Read instructions before use

Number of Tests

Store at

Use by

Keep away from heat and direct sun light

Manufacturer

EC REP — Authorized Representative in Europe