



## DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F,  
Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

[onestep@rapidtest.com](mailto:onestep@rapidtest.com)

[technicalsupport@rapidtest.com](mailto:technicalsupport@rapidtest.com)

[www.rapidtest.com](http://www.rapidtest.com)

IVD



See external label



2°C-8°C



Σ=96 tests

REF

Cat # 9061-11

# H. pylori IgG

## Chemiluminescence

Cat. 9061-11

### SUMMARY OF ASSAY PROCEDURE

Step	(20-25°C Room temp.)	Volume	Incubation time
1	Sample dilution 1:40 =5 µl / 200 µl		
2	Diluted samples, controls & calibrators	100 µl	30 minutes
3	Washing buffer (3 times)	350 µl	
4	Enzyme conjugate	100 µl	30 minutes
5	Washing buffer (3 times)	350 µl	
6	Substrate A and Substrate B mixture	100µl	5 minutes
7	Read with Luminometer in 5~30 minutes		

## NAME AND INTENDED USE

*Helicobacter pylori* IgG Chemiluminescence ELISA is intended for use in evaluating the serologic status to *H. pylori* infection in patients with gastrointestinal symptoms.

## SUMMARY AND EXPLANATION OF THE TEST

*Helicobacter pylori* is a spiral bacterium cultured from human gastric mucosa by Marshall in 1982. Studies have indicated that the presence of *H. pylori* is associated with a variety of gastrointestinal diseases including gastritis, duodenal and gastric ulcer, non-ulcer dyspepsia, gastric adenocarcinoma and lymphoma. The organism is present in 95-98% of patients with duodenal ulcer and 60-90% of patients with gastric ulcers. The studies have also demonstrated that removal of the organism by antimicrobial therapy is correlated with the resolution of symptoms and cure of diseases.

Patients who present with clinical symptoms relating to the gastrointestinal tract can be diagnosed for *H. pylori* infection by two methods:

- 1) invasive techniques include biopsy followed by culture or histologic examination of biopsy specimen or direct detection of urease activity.
- 2) non-invasive techniques include urea breath tests and serological methods.

All of the testing performed on biopsy samples are subject to errors related to sampling and interference of contaminated bacteria. *H. pylori* IgG, testing the presence of *H. pylori* specific IgG antibody is the technique of choice for serologic tests because of its accuracy and simplicity.

## PRINCIPLE OF THE TEST

Purified *H. pylori* antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the *H. pylori* IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and substrate A & substrate B mixture is added. The light generated (RLU) is proportional to the amount of IgG specific antibody in the sample. The results are read by a microwell luminometer compared in a parallel manner with calibrator and controls.

## MATERIALS PROVIDED

- |   |                   |
|---|-------------------|
| 1. Microwell Strips: purified <i>H. pylori</i> antigen coated wells | (12 x 8 wells)    |
| 2. Sample Diluent: Blue color solution.                             | 1 vial (22 ml)    |
| 3. Washing Concentrate 10x: White Cap.                              | 1 bottle (100 ml) |
| 4. Enzyme Conjugate: Red Color Solution.                            | 1 vial (12 ml)    |
| 5. Cut-off Calibrator: Yellow Cap. <i>H. pylori</i> G Index = 1.0   | (150 µl/vial)     |
| 6. Negative Control: Range stated on label. Natural Cap.            | (150 µl/vial)     |

- |  |               |
|--|---------------|
| 7. Positive Control: Range stated on label. Red Cap.                     | (150 µl/vial) |
| 8. Substrate A: H <sub>2</sub> O <sub>2</sub> in buffer. Natural bottle. | 1 vial (7 ml) |
| 9. Substrate B: Luminol in buffer. Amber bottle.                         | 1 vial (7 ml) |

## STORAGE AND STABILITY

1. Store the kit at 2 - 8 °C.
2. Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

## WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:  
The calibrator and controls contain human source components which have been tested and found nonreactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

## SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2 - 8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

## PREPARATION FOR ASSAY

1. Prepare 1x washing buffer.  
Prepare washing buffer by adding distilled or deionized water to 10x wash concentrate to a final volume of 1 liter.

2. Bring all specimens and kit reagents to room temperature

## ASSAY PROCEDURE

1. Prepare 1:40 dilutions by adding 5 µl of the samples, negative control, positive control, and calibrators to 200 µl of sample diluent. Mix well.
2. Place the desired number of coated strips into the holder.
3. Dispense 100 µl of diluted sera, calibrators, and controls into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
4. Remove liquid from all wells and repeat washing three times with washing buffer.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
7. Mix equal volume of Substrate A & Substrate B, then dispense 100 µl of this mixture to each well.
8. Read RLU with a microwell luminometer within 5~30 minutes.

## CALCULATION OF RESULTS

### Determination of Index values

1. Calculate the mean of duplicate RLU values (B).
2. Calculate the H. pylori IgG Index of each determination by dividing the mean values of each sample (B) by Cut-off calibrator mean value (C).

For example:

Sample	Well No	RLU (A)	Mean RLU (B)	INDEX B/C
Cut-off Calibrator	A1	157588	153591 (C)	1
	B1	149594		
Positive Control	C1	359296	358747	2.34
	D1	358198		
Negative Control	E1	170	202	0.001
	F1	234		
Patient Sample 1	G1	17330	16914	0.11
	H1	16498		
Patient Sample 2	A2	777930	763650	4.9
	B2	749370		

## QUALITY CONTROL

1. In order for the assay results to be considered valid the controls should be within the ranges indicated on the labels.
2. The RLU values vary with the different luminometer used.
3. Each laboratory should establish their controls at levels in low, normal and elevated ranges for monitoring assay performance. Quality control trends should be maintained to monitor batch to batch consistency.

## INTERPRETATION

### H. pylori IgG Index Interpretation

< 0.90	Negative for IgG to H. pylori
0.91 ~ 0.99	Equivocal, sample should be retested
1 ~ 2	Low positive
2 ~ 2.5	Moderate positive
> 2.5	High positive

## PERFORMANCE CHARACTERISTICS

### Sensitivity Specificity ,Accuracy:

A total of 347 patient samples were used to evaluate specificity and sensitivity of the test. H . pylori IgG test results were compared to the endoscopic biopsy findings.

	Endoscopic Biopsy				
	N	E	P	Total	
H. pylori IgG Chemiluminescence ELISA	N	134 (D)	0	2 (B)	136
	E	6	0	5	11
	P	4 (C)	0	196 (A)	200
	Total	144	0	203	347

$$\text{Sensitivity} = A / (A + B) = 196 / 198 = 99\%$$

$$\text{Specificity} = D / (C + D) = 134 / 138 = 97\%$$

$$\text{Accuracy} = (A + D) / (A + B + C + D) = 330 / 336 = 98\%$$

The comparison of H. pylori IgG test to a commercial ELISA kit results are summarized.

	Reference ELISA				
	N	E	P	Total	
H. pylori IgG Chemiluminescence ELISA	N	96 (D)	1	4 (B)	101
	E	2	2	1	5
	P	3 (C)	0	105 (A)	108
	Total	101	3	110	214

Sensitivity =  $A / (A + B) = 107 / 109 = 96\%$

Specificity =  $D / (C + D) = 96 / 99 = 97\%$

Accuracy =  $(A + D) / (A + B + C + D)$

=  $201 / 208 = 97\%$

### Expected Values:

48 random samples were determined with H. pylori IgG Chemiluminescence ELISA. The test results were computed as IgG Index using a chosen reference serum (cut off) as IgG index 1. 19 were found to be positive (39.5%), and 29 were found to be negative (60.5%).

### Precision:

The precision of the assay was evaluated by testing three different sera of eight replicates over 3 days. The intra-assay and inter-assay C.V. are summarized below:

	Negative	Low positive	Positive
Intra-assay	9.1%	8.5%	6.4%
Inter-assay	10.5%	8.9%	7.5%

## LIMITATIONS OF THE PROCEDURE

1. The assay should be used only to evaluate patients with clinical signs and symptoms suggestive of gastrointestinal disease.
2. A positive test result does not allow one to distinguish between active infection and colonization by *H. pylori*. It does not necessarily indicate that gastrointestinal disease is present.

## REFERENCE

1. Marshall, B.J. and J. R. Warren. Unidentified curved bacilli in the stomach of patients with gastritis and Peptic ulceration, Lancet 1:1311-1314, 1984.
2. Ruaws, E.A.J. and G.N.J. Tytgat. Cure of duodenal ulcer associated with eradication of *Helicobacter pylori*, Lancet 335:1233-35, 1990.

3. Perez-Perez, G.I., S.S. Wilkin, M.D. Decker and M.J. Blaswer. Seroprevalence of *Helicobacter pylori* infection in couples. J. Clin. Microbiol. 29:642-644, 1991.

Date Adopted	Reference No.
2008-05-28	DA-H. pylori IgG-2009



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**ISO 13485-2003**



Revision Date: 02/18/09