



**HELICOBACTER PYLORI**

**IGG CLASS**

**96 WELL ELISA KIT**

**PACKAGE INSERT  
INSTRUCTIONS AND INFORMATION**

Store at 2-8°C

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## **1. INTENDED USE**

This kit is an *in-vitro* laboratory assay for the determination of IgG class Antibodies against Helicobacter pylori.

## **2. INTRODUCTION**

Helicobacter pylori are spiral Gram-negative flagellated bacteria, 2-6.5um in size, which colonizes the human gastric mucosa.

The organism, found in the mucous layer, adheres to the surface mucosa epithelium of the stomach but generally does not penetrate the gastric mucosa. There is a secondary inflammatory response in the mucosa which leads to chronic active gastritis. Helicobacter pylori appears to be the primary causative agent in most cases of peptic ulcer disease.

In the developing countries the disease appears to be more prevalent in young adults with as many as 90% of the population possible having Helicobacter pylori gastritis. In developed western countries the prevalence is much lower, and the rate of acquisition much slower.

## **3. PRINCIPLE OF THE TEST**

The Helicobacter pylori IgG test is an indirect solid-phase enzyme immunoassay that detects Helicobacter specific IgG in human serum.

Helicobacter antigens (coated onto microwells) bind corresponding antibodies in patient's serum, forming an IgG-Ag complex. Anti-human IgG conjugated with the enzyme horseradish peroxidase (HRP) is added and binds to immobilized IgG.

This complex is then reacted with a specific substrate, Tetramethylbenzidine (TMB), to yield a blue colour. The intensity of the colour is proportional to the amount of VZV-specific IgG antibodies in the serum. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour.

Following termination of colour development, the absorbance is measured at 450 and reference at 620nm.

## 4. KIT COMPONENTS

1. Helicobacter pylori Coated Wells (IgG): 12 breakapart 8-well snap-off strips coated with Helicobacter pylori antigen; vacuum sealed, in resealable aluminium foil.
2. Helicobacter pylori IgG Standards\*\*\*: 4 vials containing 2mls, ready to use; blue caps  
Std A : 150 units/mL  
Std B : 75 units/mL  
Std C : 15 units/mL  
Std D : 0 units/mL
3. IgG Sample Diluent \*\*\*: 2 bottles containing 50 ml of buffer for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; amber cap.
4. Helicobacter pylori anti-IgG conjugate\*\* : 2 bottles containing 10 ml of peroxidase labelled rabbit antibody to human IgG; coloured blue, ready to use; blue cap.
5. Washing Solution (20x conc.)\*: 1 bottle containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
6. TMB Substrate Solution: 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; amber cap. Colourless solution.
7. Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; clear cap

\* contains 0.01 % Kathon after dilution

\*\* contains 0.2 % Bronidox L

\*\*\* contains 0.1 % Kathon

10. 1 Strip holder

11. 1 Package Insert

## 5. OTHER EQUIPMENT REQUIRED BUT NOT SUPPLIED

### **This protocol has been validated for the Triturus™ Analyser**

1. MICROWELL PLATE READER (capable of reading at 450 / 620 nm).
2. WASH BOTTLE - 500mL or suitable MICROWELL PLATE WASHER.
3. MEASURING CYLINDERS
4. MULTICHANNEL PIPETTE
5. REAGENT TROUGHS
6. VARIABLE VOLUME PIPETTORS (10µL – 2mL) AND DISPOSABLE TIPS
7. INCUBATOR 37°C



5. Dilute the WASH BUFFER CONCENTRATE (20x) 1/20 with distilled water. Place in either a wash bottle or plate washer reservoir.
6. Wash Procedure: Aspirate the samples from the wells and then fill all wells with diluted WASH BUFFER (350µL/well) soak for 20 seconds on the first wash. Repeat the above for 4 more cycles but with no soak time.  
NB: The washing protocol needs to be programmed for a STRIP WASH not a plate wash.
7. Add 100µL of Helicobacter pylori anti-IgG CONJUGATE to all microwells except the substrate blank.
8. Incubate the plate for 30 MINUTES ± 5 MINUTES at ROOM TEMPERATURE.
9. Wash the plate as in step 6 using a plate wash. NB no soak time is required in this wash step.
10. Add 100µL/well of TMB SUBSTRATE to all microwells. Incubate the plate at ROOM TEMPERATURE for 15 MINUTES.
11. Stop the reaction by adding 100µL/well of STOP SOLUTION to the microwells.
12. Read the plate at 450/620nm within 15mins of stopping the reaction
13. Calculate the corrected optical densities (O.D.) by subtracting the Blank O.D. from the mean O.D. of the controls and samples.

## 7. CALCULATION AND INTERPRETATION OF RESULTS

Use Linear Regression (lin/lin) to plot the standard curve using the mean of the OD's.

Read the patient results from the curve. Where patients are run in duplicate use the mean OD.

Normal value ranges should be established by each laboratory for their population.

The following values can be used as a guide :

Detected : >20 units/mL

Equivocal : 15-20 units/mL

Not detected : <15 units/mL

## 8. PRECAUTIONS

1. In compliance with article 1 paragraph 2b European directive 98/79/EC, the use of this in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analysers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.

2. All components containing material of human origin have been tested and found negative for HBsAg, anti-HCV and antibodies to HIV. They should still, however, be treated as though they were potentially infectious.
3. Do not substitute any component for the ones supplied with the kit.
4. All components that contain preservatives must have care exercised in handling/disposing of these products.
5. Heat inactivation or repeated freezing and thawing of serum samples may cause erroneous results.
6. Performing the assay at temperatures other than those stated may cause erroneous results.
7. Do not use components after the expiry date stated on the label.
8. Care should be taken to reduce microbial contamination of reusable kit components.
9. To avoid cross-contamination do not interchange screw caps of components.
10. It is recommended that external or "in-house" controls be included with each assay.

**WARNING: In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!**

**WARNING: Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!**

## **9. REAGENT STORAGE AND SHELF LIFE**

- All kit components must be stored at 2 – 8°C. All reagents are stable until labelled expiration date when stored at 2 – 8°C.

## **10. SPECIMEN COLLECTION**

- Blood should be collected by venepuncture.
- Specimens can be stored at 2-8°C for 5 days before testing. If the assay is not completed within this time then aliquot and freeze (-20 to -70°C). Avoid repeated freezing and thawing.

## **11. QUALITY CONTROL**

- The Substrate Blank and standards should be run each time the assay is performed
- Substrate blank: Absorbance value lower than 0.300.
- Standard D : Absorbance value lower than 0.400

## 12. LIMITATIONS

- As with other diagnostic test procedures, the results obtained serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
- Data from immunocompromised patients and newborns have only restricted value.
- Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values
- Interferences with haemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml haemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

## 13. TECHNICAL PERFORMANCE

### A. INTRA-ASSAY VARIATION

Sample No.	n	Mean	%CV
Pos Serum	19	0.56	6.1
Pos Serum	19	1.50	7.0

### B. INTER-ASSAY VARIATION

Sample No.	n	Mean	%CV
Pos. Serum	13	1.30	7.2
Pos. Serum	13	3.7	3.0

- The diagnostic specificity is defined as the probability of the assay scoring a negative in the absence of the specific analyte.  
It is 93.3 %.
- The diagnostic sensitivity is defined as the probability of the assay scoring a positive in the presence of the specific analyte.  
It is 93.7 %.

## 14. REFERENCES

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## **15. LIMITED EXPRESS LIABILITY**

The manufacturer makes no express warranty other than the diagnostic kit will measure IgG antibodies against *Helicobacter pylori* when used in accordance with the manufacturer's instructions. The use of the diagnostic kit for any other purpose or for the clinical diagnosis of a disease state is outside the intended use of this product.

The manufacturer disclaims any and all implied merchantability, fitness for use or implied utility for any other purpose. Any or all damages for failure of the diagnostic kit to perform according to its instructions are limited to the replacement value of the kit.

In some jurisdictions the law makes these disclaimers unenforceable and, accordingly all or part of the disclaimer may not apply to all users.

Manufactured by:  
Vital Diagnostics Pty Ltd  
38/5 Anella Avenue  
Castle Hill NSW 2154  
Australia  
P +61 (0)2 9894 6988  
F +61 (0)2 9899 6303  
ACN 003 153 286