This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1.0 INTRODUCTION
Intended Use
The Quantitative Measurement of Anti-H. Pylori Specific Antibodies of the IgG, IgA or IgM type in Human Serum or Plasma by Microplate Enzyme Immunoassay

2.0 EXPLANATION OF THE TEST
DRG's microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, diluted sample specimen, or control is first added to a microplate well. Biotinylated H. Pylori is added, and then the reactants are mixed. A reaction result between the autoantibodies to H.Pylori and the biotinylated H.Pylori to form an immune complex, which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin.
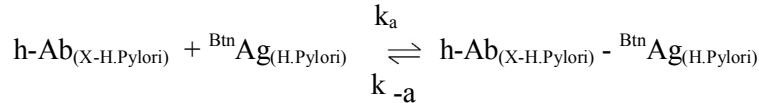
After the completion of the required incubation period, aspiration or decantation separates the reactants that are not attached to the wells. An enzyme anti-human IgG, M or A conjugate is then added to permit quantitation of reaction through interacting with human IgG, M or A of the immune complex. After washing, the enzyme activity is determined by reaction with substrate to product color. The employment of several serum references of known antibody activity permits construction of a graph of enzyme and antibody activities. From comparison to the dose response curve, an unknown specimen's enzyme activity can be correlated with autoimmune antibody level.

3.0 PRINCIPLE
A Sequential ELISA Method (TYPE 1):
The reagents required for the sequential ELISA assay include immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenous added biotinylated H. Pylori antigen.

Upon mixing biotinylated antigen, and a serum containing the antibody, reaction results between the antigen and the antibody to form an immune-complex. The interaction is illustrated by the following equation:

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$$h\text{-}Ab_{(X\text{-}H.Pylori)} + {}^{Btn}Ag_{(H.Pylori)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} h\text{-}Ab_{(X\text{-}H.Pylori)} - {}^{Btn}Ag_{(H.Pylori)}$$

${}^{Btn}Ag_{(H.Pylori)}$ = Biotinylated Antigen (Constant Quantity)

$h\text{-}Ab_{(X\text{-}H.Pylori)}$= Human Auto-Antibody (Variable Quantity)

$Ab_{(X\text{-}H.Pylori)}\text{-}{}^{Btn}Ag_{(H.Pylori)}$ = Immune Complex (Variable Quantity)

k_a = Rate Constant of Association

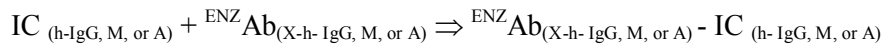k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. This interaction is illustrated below:

$h\text{-}Ab_{(X\text{-}H.Pylori)}\text{-}{}^{Btn}Ag_{(H.Pylori)}+Streptavidin_{C.W.} \Rightarrow$ immobilized complex (IC)
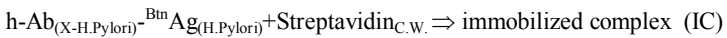
$Streptavidin_{C.W.}$ = Streptavidin immobilized on well

Immobilized complex = sandwich complex bound to the solid surface

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti-h-IgG, IgM, or IgA) is then added to the microwells. This conjugates binds to the immune complex that formed.

$IC_{(h\text{-}IgG, M, or A)} + {}^{ENZ}Ab_{(X\text{-}h\text{-} IgG, M, or A)} \Rightarrow {}^{ENZ}Ab_{(X\text{-}h\text{-} IgG, M, or A)} \text{-} IC_{(h\text{-} IgG, M, or A)}$

$IC_{(h\text{-} IgG, M, or A)}$ = Immobilized Immune complex (Variable Quantity)

${}^{ENZ}Ab_{(X\text{-}h\text{-} IgG, M, or A)}$ = Enzyme-antibody Conjugate (Constant Quantity)

${}^{ENZ}Ab_{(X\text{-}h\text{-} IgG, M, or A)} \text{-} I.C._{(h\text{-} IgG, M, or A)}$ = Ag-Ab Complex (Variable)

The anti-h-IgG, IgM or IgA enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown can be ascertained

4.0 REAGENTS
Materials provided:
A.   Anti-H.Pylori Calibrators - 1 ml/vial
Five (5) vials of references for anti-H.Pylori at levels of 0(A), 10(B), 25(C), 50(D), and 100(E) U/rnl* of the IgG, IgM or IgA type. Store at 2-8°C. A preservative has been added. 'Manufacturers' Reference Value

B.   H.Pylori Biotin Reagent - 13ml/vial
One (1) vial of biotinylated inactivated H.Pylori (IgG, IgM or IgA) in a buffering matrix. A preservative has been added. Store at 2-8°C.

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C. **H. Pylori Enzyme Reagent - 13ml/vial**
One (1) vial of anti-human IgG, IgM or IgA-horseradish peroxides (HRP) conjugate in a buffering matrix. A preservative has been added. Store at 2-8°C.

D. **Streptavidin Coated Plate - 96 wells**
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. **Serum Diluent-20ml**
One (1) vial of serum diluent containing buffer salts and a dye. Store at 2-8°C.

F. **Wash Solution Concentrate-20ml**
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C

G. **Substrate A - 7ml/vial**
One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

H. **Substrate B - 7ml/vial**
One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

I. **Stop Solution - 8ml/vial**
One (1) bottle containing a strong acid (1N HCI). Store at 2-8°C.

J. **Product Instructions.**

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8"C. Kit and component stability are identified on the label.
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 10, 25 & 50 µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological, and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin.. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (lgM & IgA) or 0.050ml (IgG) of the diluted specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the normal, borderline and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Serum Diluent
Dilute the serum diluent to 200ml in a suitable container with distilled or deionized water. Store at 2-8°C.
2. Wash Buffer
Dilute contents of wash concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
3. Working Substrate Solution
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

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4. Specimen Sample Dilution (1/100)

Dispense 0.010ml (10 µl) of each sample specimen into 1ml of serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).
Test Procedure should be performed by a skilled individual or trained professional

1. Format the microplates' wells for each serum reference, control and sample specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag. Seal and store at 2-8°C.
2. Pipette 0.025 ml (25 µl) of the appropriate serum reference, control or diluted sample specimen into the assigned well for IgG determination. For lgM or IgA, pipette 0.050ml (50 µl) of the appropriate serum reference, control or diluted sample specimen Into the assigned well.
3. Add 0.100 ml (100 µl) of H.Pylori Biotin Reagent Solution.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 350 µl of wash buffer (see Reagent Preparation Section), decant (blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's Instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times
8. Add 0.100 ml (100 µ1) of H. Pylori Enzyme Reagent to all wells. Always add reagents in the same order to minimize reaction time differences between wells.

<div align="center">

DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION

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9. Cover and incubate for thirty (30) minutes at room temperature.
10. Repeat steps (6 & 7) as explained above. Add 0.100 ml (100 µl) of Working Substrate Solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.

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DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

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12. Incubate at room temperature for fifteen (15) minutes.
13. Add 0.050ml (50 µl) of stop solution to each well and swirl the microplate gently for 15-20 seconds to mix. Always add reagents in the same order to minimize reaction time differences between wells.
14. Read the absorbance in each well at 450 nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

Note: For re-assaying specimens with concentrations greater than 100 U/ml, dilute the sample an additional 1:5 or 1:10 using the original diluted material in the serum diluent. Multiply by the dilution factor to obtain the concentration of the specimen.

10.0 CALCULATION OF RESULTS

A reference curve is used to ascertain the concentration of anti-H. Pylori in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding anti-H. Pylori activity in U/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the level of anti-H. Pylori activity for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in U/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example the average absorbance 1.603 intersects the dose response curve at 64.0 U/ml anti-H. Pylori concentration. (see Figure 1).

Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1 (Typical results for IgG, M or A)

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (U/ml)
Cal A	A1	0.042	0.044	0
	B1	0.046		
Cal B	C1	0.424	0.406	10
	D1	0.388		
Cal C	E1	0.810	0.791	25
	F1	0.772		
Cal D	G1	1.351	1.312	50
	H1	1.273		
Cal E	A2	2.377	2.328	100
	B2	2.279		
Sample 1	C2	0.163	0.172	5.2
	D2	0.182		
Sample 2	A3	1.534	1.603	64.0
	B3	1.671		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

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Figure 1.

Figure 1
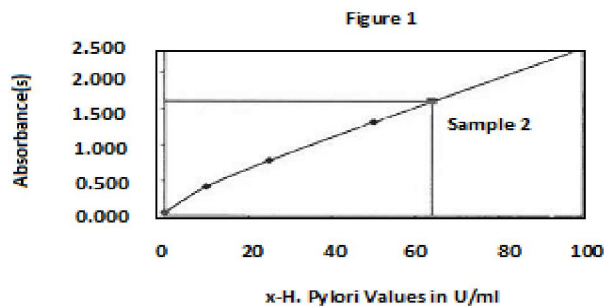
11.0 Q.C. PARAMETERS
In order for the assay results to be considered valid the following criteria should be met:
1. Maximum Absorbance (100 U/ml calibrator) = > 1.3
2. The Dose Response Curve (80%, 50%, & 20% intercepts) should be within established parameters.
3. Four out of six quality control pools should be within the established ranges.

12.0   RISK ANALYSIS
The MSDS and Risk Analysis Form for this product are available on request from DRG International, Inc.
12.1   Assay Performance
1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot No intermixing of reagents from different batches.
9. Very high concentration of anti-H. Pylori in sample specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any sample specimen with over 3.0 units of absorbance.

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10. Sample specimens with concentrations greater than 100 U/mL may be diluted (1/5 or 1/10) further than the initial 1/100 dilution using the serum diluent. The sample's concentration is obtained by multiplying the result by the dilution factor.
11. Samples, which are contaminated microbiologically, should not be used.
12. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from DRG's IFU may yield inaccurate results.
13. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
14. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
15. Risk Analysis: as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by DRG, can be requested via email from corp@drg-international.com.

13.0 REFERENCES

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