

HELICOBACTER PYLORI IgG QUANTITATIVE ENZYME IMMUNOASSAY TEST KIT

Catalog Number: EA100993



Enzyme Immunoassay for the Quantitative Determination of IgG Antibodies to *Helicobacter pylori* in Serum

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

PRINCIPLE OF THE TEST

Purified *H. pylori* antigen is coated on the surface of microwells. Diluted serum sample is added to the wells, and the *H. pylori* IgG-specific antibody, if present, binds to the antigen. All unbound materials are washed away. Enzyme conjugate is added, which binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

REAGENTS

Materials provided with the kit:

- Purified *H. pylori* antigen coated microtiter plate, 96 wells.
- Enzyme Conjugate Reagent (red color), 13 ml
- Sample Diluent (green color), 22 ml
- Low Control, < 6.25 U/ml, 100 μ L
- Standards, 0, 6.25, 12.5, 25, 50, and 100 U/ml, 100 μ L each
- High Control, > 100 U/ml, 100 μ L
- Wash Buffer (20 \times), 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

STORAGE OF TEST KITS AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Dilute 1 volume of Wash Buffer (20 \times) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20 \times) into distilled water to prepare 1000 ml of Wash Buffer (1 \times). Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Prepare 1:40 dilution for test samples, all six *H. pylori* Standards, Low Control, and High Control by adding 5 μ l of the sample to 200 μ l of sample diluent. Mix well.
3. Dispense 100 μ l of diluted sera, six standards, and controls into the appropriate wells. For the reagent blank, dispense 100 μ l sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well for 10 seconds.
4. Incubate at room temperature for 30 minutes.
5. At the end of the incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 4 times with diluted wash buffer (1 \times) and then one time with distilled water. (Please do not use tap water.)
6. Dispense 100 μ l of enzyme conjugate to each well. Mix gently for 10 seconds.
7. Incubate at room temperature for 30 minutes.
8. Remove enzyme conjugate from all wells. Rinse and flick the microtiter wells 4 times with diluted wash buffer (1 \times) and then one time with distilled water.
9. Add 100 μ l of TMB Reagent to each well. Mix gently for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Add 100 μ l of Stop Solution to each well including the 2 blanks.

TECHNICAL CONSULTATION

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- Mix gently for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
- Read the optical density at 450 nm ***within 15 minutes*** with a microtiter plate reader.

Important Note:

The wash procedure is critical. Insufficient washing will result in improper color development.

CALCULATION OF RESULTS

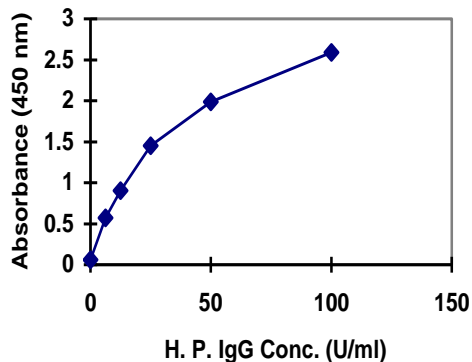
- Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls and samples.
- Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in U/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
- Use the mean absorbance values for each specimen to determine the corresponding concentration of *H. pylori* IgG in U/ml from the standard curve.

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EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against *H. pylori* IgG concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

<i>H. pylori</i> IgG (U/ml)	Absorbance (450 nm)
0	0.059
6.25	0.573
12.5	0.901
25	1.450
50	1.988
100	2.591



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