

# HELICOBACTER PYLORI IgG ENZYME IMMUNOASSAY TEST KIT

## Catalog Number: EA100992



### Enzyme Immunoassay for the Detection 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 substrate and chromogen are 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, 100  $\mu$ l
- Calibrator, *H. pylori* IgG EIA Index = 1, 100  $\mu$ l
- High Control, 100  $\mu$ l
- Wash Buffer (20 $\times$ ), 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

##### Materials required but not provided:

- Distilled water
- Precision pipettes: 5  $\mu$ l, 100  $\mu$ l and 200  $\mu$ l
- Disposable pipette tips
- Vortex mixer or equivalent
- Absorbent paper or paper towel

#### 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 a 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 of test samples, Low Control, High Control, and Calibrator by adding 5  $\mu$ l of the sample to 200  $\mu$ l of sample diluent. Mix well.
3. Dispense 100  $\mu$ l of diluted sera, Calibrator, and Controls into the appropriate wells. For the reagent blank, dispense 100  $\mu$ 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  $\mu$ 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  $\mu$ l of TMB Reagent to each well. Mix gently for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Add 100  $\mu$ l of Stop Solution to each well including the 2 blanks.

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

***Important Note:***

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

## **CALCULATION OF RESULTS**

1. Calculate the mean of duplicate calibrator value  $x_c$ .
2. Calculate the mean of duplicate High control, Low Control and samples.
3. Calculate the *H. pylori* IgG EIA Index of each determination by dividing the mean values of each sample by calibrator mean value,  $x_c$ .

## **TECHNICAL CONSULTATION**

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