Human Helicobacter pylori IgG ELISA Kit

ARG83515 Human Helicobacter pylori IgG ELISA Kit is an Enzyme Immunoassay kit for the quantification of Human Helicobacter pylori IgG in Serum, Plasma (citrate, heparin).

Catalog number: ARG83515

Package: 96 wells

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INTRODUCTION

Helicobacter pylori (H. pylori) is a spiral-shaped bacterium that grows in the mucus layer that coats the inside of the human stomach. Although many bacteria cannot survive the stomach's acid environment, H. pylori is able to neutralize the acidity of its local environment in the stomach, though not the stomach as a whole. This local neutralization helps the bacterium survive.

Another way H. pylori survives in the stomach's acidic environment is by burrowing into the mucus layer and attaching to the cells that line its inner surface. This also helps it avoid immune destruction, because even though immune cells that normally recognize and attack invading bacteria accumulate near sites of H. pylori infection, they are unable to reach the stomach lining.

H. pylori also interferes with local immune responses, making them ineffective in eliminating this bacterium.

H. pylori mainly spreads from person to person through oral contact with stool (fecal–oral), saliva (oral–oral), or vomit (gastric–oral). In most populations, the bacterium is first acquired during childhood. Infection is more likely in children living in poverty, in crowded conditions, and in areas with poor sanitation.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. Specificity Helicobacter pylori antigens has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Helicobacter pylori IgG present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated human antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C
Standards (0-3)	4 vials (0, 15, 75, 150, U/ml)(Ready-to-use)	4°C
Sample buffer	100 ml (Ready-to-use)	4°C
HRP-Antibody conjugate	20 ml (Ready-to-use)	4°C
20X Wash buffer	50 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 37°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.
- Patient sample: Dilute patient sample 1:100 with 1X sample buffer before assay, mix well. (e.g. 10 µl of sample + 990 µl of 1X sample buffer)
  Note: the controls and calibrators are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100µl** of **standards**, **controls**, **samples** and **zero controls** into wells.
3. Incubate for **60 minutes** at **37°C**.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (350µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100µl 1X HRP-antibody conjugate** into each well. Incubate for **30 minutes** at **RT**.
6. Wash as according to step 4.
7. Add **100µl** of **TMB Reagent** to each well. Incubate for **15 minutes** at **room temperature**.
8. Add **100µl of Stop Solution** to each well. Incubate for **5 minutes** at **RT**. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

INTERPRETATION OF RESULTS

Blank: <0.1

Standard 3: 0.9

Positive	> 20 U/ml	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine)
Equivocal	15-20 U/ml	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as negative.

Negative	<15 U/ml	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Rheumatoid IgG ranged from 1.39-150 U/mL. The mean MDD was 1 U/ml.

Interference

No interference has been observed with the following factors:

Hemoglobin (up to 10 mg/ml)

Triglycerides (up to 0.5 mg/mL)

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 2.47% and inter-assay precision was 10.8%.