Human Helicobacter pylori (H. pylori) IgM ELISA Kit (Direct EIA)

Catalog number: EK7066

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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Catalog Number: EK7066

Size: 96 wells/kit

Sample Type: Serum, plasma

Storage: Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles.

Intended Use

The Human Helicobacter pylori (H. pylori) IgM ELISA Kit (Direct EIA) is intended for the detection of IgM antibody to H. pylori in human serum or plasma.

Summary and Explanation

H. pylori is detectable in nearly 100% of adult patients with duodenal ulcer and about 80% of patients with gastric ulcers. An association between H. pylori and gastric cancer is confirmed. In developing countries, where most children become infected by the age of 10, gastric cancer rates are very high. In the USA and other developed countries, standards of hygiene and the increasing socioeconomic status of the population have reduced the incidence of infection, and in parallel, the rates of peptic ulcers and gastric cancer have declined. There is an excellent correlation between the clinical presentation of gastritis, the presence of H. pylori in the stomach and elevated serum H. pylori antibodies. ELISA sensitivity and specificity are 90%, and the predictive value of a negative result is very high. H. pylori-specific antibodies fall significantly after successful antibacterial therapy. Eradication of H. pylori is associated with a significant reduction in duodenal ulcer recurrence. H. pylori strains are classified into two broad groups - those that express both VacA and CagA (type I) and those that produce neither (type II). Type I strains are predominant in patients with ulcers and cancer. Up to 50% of adults are infected with H. pylori, but most of them are asymptotic and will not develop ulcers. The reason is they are infected with type II. 80-100% of patients with duodenal ulcer disease produce CagA antibodies against a 128 kd antigen compared with 60-63% of H. pylori-infected persons with gastritis only, indicating that serologic responses to the 128 kd protein are more prevalent among H. pylori-infected persons with duodenal ulcers than infected persons without peptic ulceration. In H. pylori-infected patients who develop gastric cancer, antibodies against CagA are 94% sensitive and 93% specific, indicating that detection of antibodies to CagA is a useful marker for diagnosis of duodenal ulcer and gastric cancer.

Principle of the Test

Diluted patient serum (serum diluent contains sorbent to remove rheumatoid factor and human IgG interference) is added to wells coated with purified antigen. IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample.

Kit Components

Description	Quantity
1. Microwells coated with H. pylori antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml
3. Calibrator: 1 Vial (ready to use)	1ml

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4. Positive Control: 1 vial (ready to use)	1ml
5. Negative Control: 1 vial (ready to use)	1ml
6. Enzyme conjugate: 1 bottle (ready to use)	12ml
7. TMB Substrate: 1 bottle (ready to use)	12ml
8. Stop Solution: 1 bottle (ready to use)	12ml
9. Wash concentrate 20X: 1 bottle	25ml

Materials Required, but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Storage and Stability

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

Warnings and Precautions

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
4. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
5. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
6. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
7. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

Specimen Collection and Handling

1. Collect blood specimens and separate the serum.
2. Typically, specimens may be refrigerated at 2-8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

Reagent Preparation

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

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Assay Procedure

Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.
1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10ul of the sample to 200ul of sample diluent. Mix well.
3. Dispense 100ul of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100ul sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300ul of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100ul of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300ul of 1X wash buffer. Blot on absorbance paper or paper towel
7. Dispense 100ul of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100ul of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

Calculation of Results

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Limitations of the Test

1. To enhance sensitivity and specificity of this IgM test, provided sample diluent has been formulated to block IgG and Rheumatoid Factor (RF) interferences. Turbidity could be seen after diluting serum with sample diluent. This turbidity is due to the blocking of serum IgG and shows no interference with test results. It can be removed by centrifugation.
2. In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely.
3. Lipemic or hemolyzed samples may cause erroneous results.

References

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2. Holtmann G; Talley NJ; Mitchell H; Hazell S. Antibody response to specific H. pylori antigens in functional dyspepsia, duodenal ulcer disease, and health. Am J Gastroenterol 1998; 93(8):1222-7.
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4. Klaamas K; Held M; Wadstr¨om T; Lipping A; Kurtenkov O. IgG immune response to Helicobacter pylori antigens in patients with gastric cancer as defined by ELISA and immunoblotting. Int J Cancer 1996; 67(1):1-5.
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