Helicobacter pylori Antigen

Enzyme immunoassay for the qualitative determination of Helicobacter pylori antigen in human stool

ORDER INFORMATION

REF	Kit size
HP0024K	24 tests

INDICATION

The Helicobacter pylori (Hp) is a Gram Negative bacteria which was isolated for the first time by Marshall and Warren in 1983 in gastric mucosa. The bacteria, and in particular its virulent strains, are generally recognized as the causative agent and/or cofactor in gastrointestinal pathologies such as duodenal ulceration, peptic ulcers and gastritis. The bacteria has been isolated in different biological samples (saliva, vaginal mucosa, gastric juice etc.) and in food samples, contaminated by direct contact.
The diagnosis for the presence of the Helicobacter pylori antigen (Hp Ag) in the gastric mucosa can be achieved non invasively by the urea breath test and/or invasively by an upper gastrointestinal endoscopy in which a small mucosal biopsy is removed for a microscopic examination and for bacterial culturing.
The present kit is an ELISA test for the search of *H. pylori* antigen in stools. The employment of high specific monoclonal antibodies makes this kit a powerful non invasive tool for diagnosing *H. pylori* infection, with high sensitivity and specificity.
The kit can be utilized for the patients' follow up and to evaluate the efficacy of the therapy.

PRINCIPLE OF THE ASSAY

Microtiter strip wells are pre-coated with a monoclonal antibody against *H. pylori*. Diluted stool samples and controls are pipetted into the wells and incubated with monoclonal antibody cojugated to horseradish peroxidase for 90 minutes. The fecal *H. pylori* antigen will be sandwiched between the solid phase and enzyme-linked antibody. After washing the wells, the immune complex formed by the bound conjugate is visualized with TMB substrate which gives a blue colour. The stop solution ends the reaction and turns the blue colour to yellow. The colour intensity is directly proportional to the amount of faecal antigen present in the sample.

KIT CONTENT

1. **Reagent A – Microwell strips**
   Strips coated with monoclonal antibody MoAb, breakaway microwells. The strips are contained in a sealed bag with desiccant. Bring the strips to room temperature before use, to prevent any moisture formation inside the bag.
   Ref HP0024K 3 strips

2. **Reagent B – Enzymatic Tracer**
   Stabilized proteic red buffer solution containing monoclonal antibody conjugated with Horseradish peroxidase (HRP). Ready for use.
   Ref HP0024K 4 ml

3. **Reagent C – Washing Solution 5x**
   Concentrated (20x) TRIS-HCl buffer containing a detergent and sodium merthiolate. Dilute before use.
   Ref HP0024K 30 ml

4. **Reagent D/E – Chromogen-Substrate**
   Vial containing buffered tetramethyl-benzidine (TMB) and hydrogen peroxide (H_2O_2) with activators and stabilizers. Ready for use.
   Avoid light exposure.
   Ref HP0024K 8 ml

5. **Reagent F – Stop Solution**
   Vial containing HCl 2N acid solution.
   The reagent is **irritant**: x_i R36/38; S2/26/30.
   Handle with care.
   Ref HP0024K 5 ml

6. **Reagent G – Sample Diluent**
   Vial containing bovine proteins, a detergent and 7 mM NaN_3.
   Ref HP0024K 30 ml

7. **Negative Control**
   Vial containing bovine proteins, a detergent and 7 mM NaN_3.
   Ref HP0024K 1 ml

8. **Positive Control**
   Vial containing *H. pylori* antigens derived from a cultured *H. pylori* cells (strain ATCC 1989: 1 µg/mL) and 7 mM NaN_3.
   Ref HP0024K 1 ml

9. **Stirrer:** to collect and disperse stool.
   Ref HP0024K n° 24

10. **Dose pipettes**: to dispense of stool dilutions into microwells.
    Ref HP0024K n° 24

11. **Cardboard sealers**
    2 cardboard sealers to be used to cover strips during the incubations.

12. **Package insert**: instruction for use HP0024K it/ing.

MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HBsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially controls and samples. All objects come in direct contact with samples and all residuals of the assay should be treated or eliminated as potentially infected. Best procedures for inactivation are treatments with autoclave at 121 °C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 24 hours.
2. Avoid any contact with skin and mucous membrane, in particular for Stop Solution.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, use only the Washing Solution provided in the kit and follow carefully the indications reported in "WASHING INSTRUCTION".
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.

STORAGE AND STABILITY

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before re-store at 2-8 °C. After opening the bag, strips are stable up to 2 months.
3. The diluted washing solution can be stored for 2 weeks at 2-4 °C.
4. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

AUXILIARY MATERIALS

- Deionised or distilled water
- Clean test tubes for diluted samples
- Precision pipettes with disposable tips for delivering 50, and 100 µl
- Vortex and timer
- Absorbent paper
- Graduated cylinder for washing buffer dilution
- Automated or manual washing device capable of aspiration and delivering volumes of 300 - 400 µl
- ELISA reader equipped with 450 nm and 630 nm filters

SAMPLES

Human stool.

Samples must be stored at 2–4 °C for 24 hours or, if they cannot be tested within 24 hours, should be stored at -20 to -80°C.

It is suggested to thaw the samples only one time.

SAMPLE PREPARATION

It is suggested to use the extraction kit Ref HP0024EXT.
Alternatively, follow these instructions.

- Add 0.500 ml of *Sample Diluent* to a test tube.
- With the provided stirrer transfer a pea-sized sample of stool.
- Put the sample in the tube and dissolve it in the buffer. Then vortex to ensure complete re-suspension and homogenization of the sample. (Note: Stool suspension may be stored for 24 h at 2-4 °C).

REAGENTS PREPARATION

- WASHING SOLUTION: dilute 1:20 with distilled or deionised water. Mix carefully before use.

WASHING INSTRUCTION

Proper washing of the micro wells is critical to assure accurate and precise test results.

The use of a correctly maintained and adjusted automated microplate wash device is recommended. We recommend to calibrate the washing system on the kit itself so to match the declared analytical performances. Performing 6 wash cycles of 300 µl/micro well and maintaining 30 seconds intervals before aspiration of wash buffer are required to avoid high backgrounds and/or false positive results.

Tap micro wells onto absorbent paper to remove all remaining wash solution before adding next reagent.

ASSAY PROCEDURE

1. At least one hour before use, bring all reagents, controls and samples to room temperature (18-30 °C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. Distribution and incubation times must be the same for all wells in the same analysis.
4. Avoid long interruptions between each step of the assay procedure.
5. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
6. The colour developed in the last incubation is stable for a maximum of 30 minutes. Otherwise, in case of reading after 10-15 min after dispensing stop solution, immediately place the strips **in the dark**.
7. We recommend to read the plate with an ELISA automatic reader able to subtract the background at 620-630 nm and to read the absorbance of samples and controls at 450 nm.

ASSAY SCHEME

Use the Dose Pipettes provided with the kit to dispense each stool suspension (2 drops/well are about 100 µl), the Precision Pipettes to dispense Controls and other reagents.

		Anti-Hp antibody coated wells		
	REAGENTS	**Blank**	**Controls**	**Samples**
First incubation	Controls	-	100 µl	-
	Samples	-	-	100 µl
	Enzymatic Tracer	-	100 µl	100 µl
	- Gently shake micro wells for 30 seconds - Cover the strips with cardboard sealer - Incubate **90 (± 10) minutes at room temperature (18-30 °C)**			
Wash	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 6 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid			
Colorimetric reaction	Chromogen/Substrate	200 µl	200 µl	200 µl
	- Cover the strips with cardboard sealer - Incubate **15 minutes at room temperature (18-30 °C)**, avoiding light exposure			
	Reagent F (Stop Solution)	100 µl	100 µl	100 µl
	- Mix gently for 30 seconds and read the absorbance of each well at 450 (and 620-630 nm) against Blank			

QUALITY CONTROL

Results are considered valid if:

1. Mean OD values at 450nm for the Negative Control are <0.160 (colourless). Higher OD's may indicate a contamination of the Chromogen-Substrate, or insufficient washing of micro wells between procedural steps.
2. Mean OD values at 450nm for the Positive Control are >1.000 (deep yellow). Lower OD values may indicate improper storage or contamination of reagents and/or procedural errors.

If the test run is considered invalid, recheck reagent preparation and assay procedure, storage condition and expiry date of reagents before repeating assay.

CALCULATION OF RESULTS

To determine Hp antigen concentrations in test samples a **Cut Off value is used with OD 0.160** (OD 450 nm).

- Samples with OD values ≤ 0.160 are considered negative.
- Samples with OD values within 0.130-0.180 are considered border line.
- Samples with OD values > 0.160 are considered positive.

Note: Border line samples should be repeated with a confirmatory test and positive samples should be followed in the time.

ANALYTICAL PERFORMANCES

The present kit has been clinically evaluated with 55 patients undergoing gastroscopy (1). *H. pylori* infection was assessed by histology, rapid urease test, ^{13}C UBT, serology and by MGD kit. As a gold standard for *H. pylori* infection was considered the positivity of at least three tests.

Precision

The CV values are about 4-8%.

Sensitivity and Specificity

With these criteria, MGD kit showed a sensitivity of 96% and a specificity of 98%.

LIMITATION OF THE PROCEDURE

Antibiotics, protonic pump inhibitors and bismuth preparations are known to be drugs which can interfere with Hp and their use may falsify results of various diagnostic methods by exhibiting false negative samples.

PRECAUTIONS IN USE

Reagent F is **irritant (Xi)**. Refer to Safety Data Sheet.

The other reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

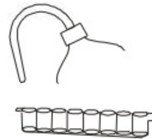
1- Dore M.P., Wengler G., Tadeu V., Marras L. Maraglkoudakis E., Osato D.Y. Graham G. Realdi D. A novel monoclonal antibody test to detect *H.pylori* antigens in human stool. The 15th International Workshop on Gastrointestinal Pathology and *Helicobacter*, Athens, Greece, 11-14 September 2002.
2- Suerbaum S, Michetti P. *Helicobacter pylori* infection. N Engl J Med. 2002;347:1175-86.
3- Sipponen P. Gastric cancer: pathogenesis, risks, and prevention. J Gastroenterol. 2002;37 Suppl 13:39-44.
4- Vaira D, Malfertheiner P, Megraud F et al.. Diagnosis of *Helicobacter pylori* infection with a new non-invasive antigen-based assay. HpSA European study group. Lancet. 1999;354:30-3.
5- Gisbert JP, Pajares JM. Diagnosis of *Helicobacter pylori* infection by stool antigen determination: a systematic review. Am J Gastroenterol. 2001;96:2829-38.

Dissolve a pea sized stool sample (approx.0.1g) in 0.5 ml of sample buffer.

Vortex for 15-20 seconds to ensure thorough mixing of the specimen.

Add two drops of each stool specimen plus 100 μl positive and negative controls to separate wells.

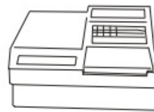
Add 100 μl of antibody conjugate to all wells. Incubate for 90 minutes.



Wash by completely filling all wells (5 times)

Add 200 μl of substrate solution (TMB) to all wells and incubate for 15 minutes

Add 100 μl of Stop solution to all wells.



Read visually or spectrophotometrically at 450 nm (single wavelength) or 450/620 (dual wavelength)

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Edition: 2010/11/23