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**User's Manual**

# Helicobacter pylori Antigen ELISA

REF **DEIABL338M**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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# PRODUCT INFORMATION

## Intended Use

This Immundiagnostik AG assay is a qualitative sandwich ELISA for determination of Helicobacter pylori in stool.

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

## General Description

Helicobacter pylori is regarded as a causative factor for chronic B-gastritis, drug-unrelated ulcus duodeni, and as an etiologic stimulus of gastric MALT-lymphoma. Furthermore, it is suspected of being involved in the pathogenesis of stomach carcinoma.

## Principles of Testing

A microtiter plate is coated with antibodies specific for Helicobacter pylori. The Helicobacter pylori antigen in the sample is bound by the immobilised antibodies during the first incubation step. At the same time, a peroxidase-labelled antibody binds the Helicobacter pylori antigen. After a washing step tetramethylbenzidine is used as substrate for peroxidase. Finally, an acidic stop solution is added to terminate the reaction. The colour changes from blue to yellow. The intensity of the colour is proportional to the amount of analyte (sample or control). The results are evaluated by comparison with a cut-off value.

## Reagents And Materials Provided

1. PLATE, Microtiter plate, pre-coated, 12 × 8 wells
2. 10×WASHBUF, 10×Wash buffer concentrate, 2 × 100 ml
3. SAMPLEBUF, Sample dilution buffer, 1 × 100 ml
4. CTRL NEG, Control negative, 1 × 1 ml
5. CTRL POS, Control positive, 1 × 1 ml
6. CONJ Conjugate, 1 × 8 ml
7. SUB Substrate (tetramethylbenzidine), 1 × 15 ml
8. STOP, Stop solution, 1 × 15 ml

## Materials Required But Not Supplied

1. Ultrapure water*
2. Laboratory balance
3. Calibrated precision pipettors and 10–1 000 µl single-use tips
4. Foil to cover the microtiter plate
5. Multi-channel pipets or repeater pipets
6. Centrifuge, 3 000 g

7. Vortex
8. Standard single-use laboratory glass or plastic vials, cups, etc.
9. Microtiter plate reader (required filters see chapter 7)

***CD recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).**

## Storage

2-8°C

## Specimen Collection And Preparation

**Sample storage**

Raw stool is stable for 7 days at room temperature, 7 days at 2–8 °C, and 7 months at -20 °C. More than 3 freeze-thaw cycles are to be avoided.

Stool extract is stable for 7 days at 2–8 °C.

**Extraction of stool samples**

Add 500 µl sample dilution buffer (SAMPLEBUF) to a stool sample of 100 mg (100 µl liquid stool sample)

OR

add 1 ml sample dilution buffer (SAMPLEBUF) to a stool sample of 200 mg (200 µl liquid stool sample) and homogenise thoroughly on a vortex mixer.

Centrifuge the suspension for 10 min at 3 000 rpm.

For analysis, pipet 50 µl supernatant of this stool extract per well.

## Reagent Preparation

**1. Preparation of the wash buffer:** The wash buffer concentrate (WASHBUF) has to be diluted with ultrapure water 1:10 before use (e. g. 100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The WASHBUF can be used until the expiry date stated on the label when stored at 2–8 °C. Wash buffer (1:10 diluted WASHBUF) can be stored in a closed flask at 2–8 °C for 1 month.

**2. All other test reagents are ready-to-use.** Test reagents can be used until the xpiry date (see label) when stored at 2–8 °C.

## Assay Procedure

**Note:**

Bring all reagents and samples to room temperature (15–30°C) and mix well.

Mark the positions of controls and samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until the expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform.

We recommend to carry out the tests in duplicate.

**Procedure:**

1. Before use, wash the wells 5 times with 250 µl wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2. Add 50 µl of conjugate (CONJ) into each well.
3. Add 50 µl positive control, negative control, and supernatant of stool extract (CTRL POS/CTRL NEG/SAMPLE) into respective well, mix shortly.
4. Cover the strips and incubate for 1 hour at room temperature (15–30 °C).
5. Discard the content of each well. Wash 5 times by dispensing 250 µl wash buffer into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper.
6. Add 100 µl substrate (SUB) into each well.
7. Incubate for 10–20 min* at room temperature (15–30 °C) in the dark.
8. Add 100 µl stop solution (STOP) into each well and mix well.
9. Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference.

*** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.**

## Interpretation Of Results

Cut-off value = 0.150 OD

The results are categorised according to their absorbances using the cut-off value ± 0.020 OD as criterion:

OD 0.130–0.170 (cut-off value ± 0.020 OD) borderline

OD >0.170 (> cut-off value + 0.020 OD) positive

OD <0.130 (< cut-off value – 0.020 OD) negative

**QUALITY CONTROL**

CD recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

## Precision

**Repeatability (Intra-Assay); n = 22**

The repeatability was assessed with 2 stool samples under constant parameters (same operator, instrument, day and kit lots).

**Reproducibility (Inter-Assay); n = 272**

The reproducibility was assessed with 3 samples under varying parameters (different operators, instruments, days and kit lots).

## Precautions

1. All reagents in the kit package are for research use only.
2. Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide or ProClin are hazardous to health and the environment. Substrates for enzymatic colour reactions may also cause skin and/or respiratory irritation. Any contact with the substances must be avoided.
3. The 10× Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact. Warning: Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: get medical Advice/attention.
4. The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

**TECHNICAL HINTS**

1. Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
2. Control samples should be analysed with each run.
3. Reagents should not be used beyond the expiration date stated on kit label.
4. Substrate solution should remain colourless until use.
5. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
6. Avoid foaming when mixing reagents.
7. Do not mix plugs and caps from different reagents.
8. The assay should always be performed according to the enclosed manual.

**GENERAL NOTES ON THE TEST AND TEST PROCEDURE**

1. The guidelines for laboratories should be followed.
2. Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. CD can therefore not be held responsible for any damage resulting from incorrect use.

## Limitations

Samples which cannot be clearly interpreted (e. g. because of high coefficients of variation of replicates) should be measured again.