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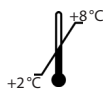
**Manual**

# **IDK<sup>®</sup> Helicobacter pylori antigen ELISA**

*For the in vitro determination of  
Helicobacter pylori in stool*

Valid from 2023-03-22

**REF** K 6923



**IVD**



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## Table of Contents

<b>1. INTENDED USE</b>	<b>13</b>
<b>2. INTRODUCTION</b>	<b>13</b>
<b>3. MATERIAL SUPPLIED</b>	<b>13</b>
<b>4. MATERIAL REQUIRED BUT NOT SUPPLIED</b>	<b>14</b>
<b>5. STORAGE AND PREPARATION OF REAGENTS</b>	<b>14</b>
<b>6. STORAGE AND PREPARATION OF SAMPLES</b>	<b>14</b>
<b>7. ASSAY PROCEDURE</b>	<b>15</b>
<i>Principle of the test</i>	15
<i>Test procedure</i>	15
<b>8. RESULTS</b>	<b>16</b>
<b>9. LIMITATIONS</b>	<b>16</b>
<b>10. QUALITY CONTROL</b>	<b>16</b>
<b>11. PERFORMANCE CHARACTERISTICS</b>	<b>17</b>
<i>Accuracy – Precision</i>	17
<i>Sensitivity and specificity</i>	17
<b>12. PRECAUTIONS</b>	<b>18</b>
<b>13. TECHNICAL HINTS</b>	<b>18</b>
<b>14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE</b>	<b>19</b>
<b>15. REFERENCES</b>	<b>19</b>

## 1. INTENDED USE

This Immundiagnostik AG assay is a sandwich ELISA for determination of *Helicobacter pylori* in stool. For *in vitro* diagnostic use only.

## 2. INTRODUCTION

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

The epidemiology of infection by *Helicobacter pylori* has been characterised in western industrial nations by a linear increase with age. In developing countries a large number of children and juveniles is affected.

The currently used methods for the diagnosis of *Helicobacter pylori* infection are very sensitive and highly specific, but all include either invasive sampling or require special technical devices.

We have developed an ELISA for the detection of *Helicobacter pylori* infection from faecal samples. This cost-efficient methodology provides a reliable result without the loss of sensitivity or specificity and does not require invasive sampling.

### Indications

- Detection of a *Helicobacter pylori* infection
- Monitoring the effect of a *Helicobacter pylori* treatment

## 3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6923	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10x	2 x 100 ml
K 6923	SAMPLEBUF	Sample dilution buffer; ready-to-use	1 x 75 ml
K 6923	CTRL NEG	Control negative, ready-to-use	1 x 1 ml
K 6923	CTRL POS	Control positive, ready-to-use	1 x 1 ml
K 6923	CONJ	Conjugate, ready-to-use	1 x 8 ml
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as purchase order number.

## 4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water\*
- Laboratory balance
- Calibrated precision pipettors and 10–1 000 µl single-use tips
- Foil to cover the microtiter plate
- Multi-channel pipets or repeater pipets
- Centrifuge, 3 000 g
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

\* Immundiagnostik AG 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).

## 5. STORAGE AND PREPARATION OF REAGENTS

- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (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**.
- All other test reagents are ready-to-use. Test reagents can be used until the expiry date (see label) when stored at **2–8 °C**.

## 6. STORAGE AND PREPARATION OF SAMPLES

### 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.

Immundiagnostik AG recommends for sample preparation the use of Roche Diagnostics / Mannheim sample preparation tubes, article No. 10745804322.

## 7. ASSAY PROCEDURE

### *Principle of the test*

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.

### *Test procedure*

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. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	<b>Before use</b> , wash the wells <b>5 times</b> with <b>250 µl wash buffer</b> . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add <b>50 µl of conjugate</b> (CONJ) into each well.
3.	<b>Add 50 µl positive control, negative control, and supernatant of stool extract</b> (CTRL POS/CTRL NEG/SAMPLE) into respective well, mix shortly.
4.	Cover the strips and incubate for <b>1 hour</b> at room temperature (15–30°C).

5.	Discard the content of each well. Wash <b>5 times</b> by dispensing <b>250 µl</b> wash buffer into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper.
6.	Add <b>100 µl substrate</b> (SUB) into each well.
7.	Incubate for <b>10–20 min*</b> at room temperature (15–30 °C) in the <b>dark</b> .
8.	Add <b>100 µl stop solution</b> (STOP) into each well and mix well.
9.	Determine <b>absorption immediately</b> with an ELISA reader at <b>450 nm</b> 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.

## 8. RESULTS

**Cut-off value** = 0.150 OD

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

<b>OD 0.130–0.170</b> (cut-off value $\pm 0.020$ OD)	borderline
<b>OD &gt;0.170</b> (> cut-off value + 0.020 OD)	positive
<b>OD &lt;0.130</b> (< cut-off value – 0.020 OD)	negative

## 9. LIMITATIONS

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

## 10. QUALITY CONTROL

Immundiagnostik 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 patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

## 11. PERFORMANCE CHARACTERISTICS

### *Accuracy – Precision*

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

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

Sample	Mean value [OD]	CV [%]
1	0.708	5.1
2	0.172	4.0

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

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

Sample	Mean value [OD]	CV [%]
1	0.519	7.0
2	0.354	7.1
3	0.265	7.3

### *Sensitivity and specificity*

**Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the K6920 test**

K 6920	%
Clinical sensitivity	97.7
Clinical specificity	96.3
Positive predictive value (PPV)	98.8
Negative predictive value (NPV)	92.9

Samples, n = 113; *H. pylori* positive, n = 86; *H. pylori* negative, n = 27  
Comparative measurements with K 6920 and K 6923 (Samples, n = 45; *H. pylori* positive, n = 30; *H. pylori* negative, n = 15) showed a 100% match between the values.

## 12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- 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. Further safety information can be found in the safety data sheet, which is available from Immundiagnostik AG on request.
- The 10x 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.

- 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.

## 13. TECHNICAL HINTS

- 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.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.















## 14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- *IDK*® is a trademark of Immundiagnostik AG.
- 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. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

## 15. REFERENCES

1. S. Blanco et al., 2008. Comparison of stool antigen immunoassay methods for detecting *Helicobacter Pylori* infection before and after eradication treatment. *Diagnostic Microbiology and Infectious Disease* **61** pp. 150–155.

**Used symbols:**

	Temperature limitation		Catalogue number
	In Vitro Diagnostic Medical Device		To be used with
	Manufacturer		Contains sufficient for <n> tests
	Lot number		Use by
	Attention		Consult instructions for use
	Consult specification data sheet		Irritant