

# Helicobacter pylori IgM ELISA

Enzyme immunoassays for the qualitative and quantitative determination of IgM antibodies against Helicobacter pylori in human serum and plasma.

**REF**      **RE56391**

      **12x8**

        **2-8°C**

EU: **IVD** 



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## 1. INTENDED USE

Enzyme immunoassay for the qualitative and quantitative determination of IgM antibodies against Helicobacter pylori in human serum and plasma.

## 2. SUMMARY AND EXPLANATION

Helicobacter pylori is a 2.5-3 µm long twisted or helical gram-negative germ responsible for 80-90 % of B-gastritis cases and suspected to a major cofactor for the development of gastric and duodenal ulcers. The classical detection methods of Helicobacter pylori like culturing from mucous membrane biopsies or an urease test are only successful in case of a relative high germ count and require an identification directly after the biopsy. Indirect immunofluorescence (IIF) is also used for the detection Helicobacter pylori infection.

The colonisation of the gastric and duodenal mucous membranes with Helicobacter pylori can also be detected serologically using an enzyme immunoassay (ELISA) or by performing a Western Blot. Patients with confirmed exposition to Helicobacter pylori often show a positive serological result. Since antibodies persist for a longer time after a Helicobacter pylori infection seropositive individuals are also found in symptom-free patients. The number of seropositive values rises with age.

Using the ELISA and the detection of IgA and IgG antibodies against specific proteins of Helicobacter pylori by Western Blotting it is possible to diagnose an acute infection with Helicobacter pylori, even if no germs can be found.

## 3. TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with antigen. Specific antibodies of the sample binding to the antigen coated wells are detected by a secondary enzyme conjugated antibody (E-Ab) specific for human IgM. After the substrate reaction the intensity of the color developed is proportional to the amount of IgM-specific antibodies detected. Results of samples can be determined directly using the standard curve.

## 4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. Some reagents contain sodium azide (NaN<sub>3</sub>) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN<sub>3</sub> may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
10. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

## 5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8 °C.

## 6. SPECIMEN COLLECTION AND STORAGE

### Serum, Plasma (EDTA, Heparin)

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8 °C	-20 °C	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.
Stability:	2 days	> 2 days	

## 7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	<b>MTP</b>	<b>Microtiter Plate</b> Break apart strips. Coated with specific antigen.
1 x 15 mL	<b>ENZCONJ IgM</b>	<b>Enzyme Conjugate IgM</b> Red colored. Ready to use. Contains: anti-human IgM, conjugated to peroxidase, protein-containing buffer, stabilizers.
1 x 4 x 2 mL	<b>CAL A-D</b>	<b>Standard A-D</b> 1; 10; 25; 150 U/mL. Ready to use. Standard A = Negative Control Standard B = Cut-Off Control Standard C = Weakly Positive Control Standard D = Positive Control Contains: IgM antibodies against Helicobacter pylori, PBS, stabilizers.
1 x 15 mL	<b>TMB SUBS</b>	<b>TMB Substrate Solution</b> Ready to use. Contains: TMB.
1 x 15 mL	<b>TMB STOP</b>	<b>TMB Stop Solution</b> Ready to use. 0.5 M H <sub>2</sub> SO <sub>4</sub> .
1 x 60 mL	<b>DILBUF</b>	<b>Diluent Buffer</b> Ready to use. Contains: PBS Buffer, BSA, < 0.1 % NaN <sub>3</sub> .
1 x 60 mL	<b>WASHBUF CONC</b>	<b>Wash Buffer, Concentrate (10x)</b> Contains: PBS Buffer, Tween 20.
2 x	<b>FOIL</b>	<b>Adhesive Foil</b> For covering of Microtiter Plate during incubation.
1 x	<b>BAG</b>	<b>Plastic Bag</b> Resealable. For dry storage of non-used strips.

## 8. MATERIALS REQUIRED BUT NOT SUPPLIED

- RF Absorbent (can be ordered separately from IBL under **REF** KIRF561)
- Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volumes: 5; 50; 100; 500 µL
- Calibrated measures
- Tubes (1 mL) for sample dilution
- 8-Channel Micropipettor with reagent reservoirs
- Wash bottle, automated or semi-automated microtiter plate washing system
- Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
- Bidistilled or deionised water
- Paper towels, pipette tips and timer

## 9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Use a pipetting scheme to verify an appropriate plate layout.
5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
7. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

## 10. PRE-TEST SETUP INSTRUCTIONS



In order to avoid interferences of specific IgG and rheumatoid factors, patient sera should be treated with RF absorbent (REF KIRF561).

### 10.1. Preparation of Components



The contents of the kit for 96 determinations can be divided into 3 separate runs. The volumes stated below are for one run with 4 strips (32 determinations).

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
20 mL	WASHBUF CONC	200 mL	bidist. water	1:11	Warm up at 37 °C to dissolve crystals, if necessary. Mix vigorously.	2-8 °C	8 w
1 mL	RF-Absorbent	20 mL	DILBUF	1:21	Incubate ≥ 1 min.	2-8 °C	8 w

### 10.2. Dilution of Samples

Sample	to be diluted	with	Relation	Remarks
Serum / Plasma	generally	DILBUF (+ RF-Absorbent)	1:101	e.g. 5 µL + 500 µL DILBUF

Samples containing concentrations higher than the highest standard have to be diluted further.

Samples with RF-Absorbent: Do not incubate >20 min to avoid adsorption of specific antibodies.

Pretreated samples may be turbid.

## 11. TEST PROCEDURE

1.	Pipette <b>100 µL</b> of each <b>Standard and diluted sample</b> into the respective wells of the Microtiter Plate. In the qualitative test only Standard B is used.
2.	Cover plate with adhesive foil. <b>Incubate 60 min at 18-25 °C.</b>
3.	Remove adhesive foil. Discard incubation solution. Wash plate <b>3 x</b> with <b>300 µL</b> of <b>diluted Wash Buffer</b> . Remove excess solution by tapping the inverted plate on a paper towel.
4.	Pipette <b>100 µL</b> of <b>Enzyme Conjugate</b> into each well.
5.	Cover plate with new adhesive foil. <b>Incubate 30 min at 18-25 °C.</b>
6.	Remove adhesive foil. Discard incubation solution. Wash plate <b>3 x</b> with <b>300 µL</b> of <b>diluted Wash Buffer</b> . Remove excess solution by tapping the inverted plate on a paper towel.
7.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
8.	Pipette <b>100 µL</b> of <b>TMB Substrate Solution</b> into each well.
9.	<b>Incubate 20 min at 18-25 °C</b> in the dark (without adhesive foil).
10.	Stop the substrate reaction by adding <b>100 µL</b> of <b>TMB Stop Solution</b> into each well. Briefly mix contents by gently shaking the plate. Color changes from blue to yellow.
11.	<b>Measure</b> optical density with a photometer at <b>450 nm</b> (Reference-wavelength: 600-650 nm) within <b>60 min</b> after pipetting of the Stop Solution.

## 12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards/controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

## 13. CALCULATION OF RESULTS

The evaluation of the test can be performed either quantitatively or qualitatively.

### 13.1. Qualitative Evaluation

The Cut-off value is given by the optical density (OD) of the Standard B (Cut-off standard). The Cut-off index (COI) is calculated from the mean optical densities of the sample and Cut-off value. If the optical density of the sample is within a range of 20 % around the Cut-off value (grey zone), the sample has to be considered as borderline. Samples with higher ODs are positive, samples with lower ODs are negative.

For a quantification, the Cut-off index (COI) of the samples can be formed as follows:

$$\text{COI} = \frac{\text{OD Sample}}{\text{OD Standard B}}$$

### 13.2. Quantitative Evaluation

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline or point-to-point curve, because these methods give the highest accuracy in the data calculation.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

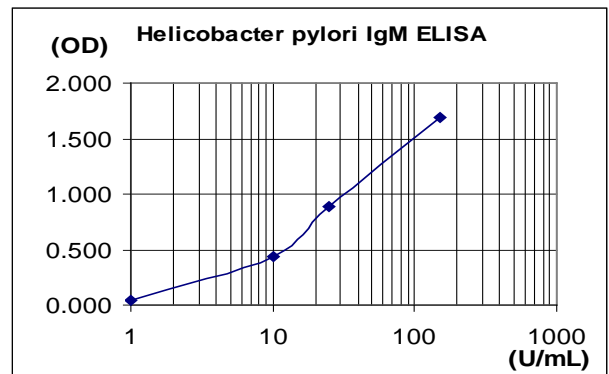
The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

### Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	U/mL	OD <sub>Mean</sub>
A	1	0.043
B	10	0.440
C	25	0.890
D	150	1.697



## 14. INTERPRETATION OF RESULTS

Method	Range	Interpretation
Quantitative (Standard curve)	< 8 U/mL	negative
	8 – 12 U/mL	equivocal
	> 12 U/mL	positive
Qualitative (Cut-off Index, COI)	< 0.8	negative
	0.8 – 1.2	equivocal
	> 1.2	positive

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

## 15. EXPECTED VALUES

In an in-house study, apparently healthy subjects showed the following results:

Ig Isotype	n	Interpretation		
		positive	equivocal	negative
IgM	72	1.4 %	2.8 %	95.8 %

## 16. LIMITATIONS OF THE PROCEDURE

Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

For cross-reactivities, see PERFORMANCE.

Azide and thimerosal at concentrations > 0.1 % interfere in this assay and may lead to false results.

The following blood components do not have a significant effect (+/- 20 % of expected) on the test results up to the below stated concentrations:

Hemoglobin	8.0 mg/mL
Bilirubin	0.3 mg/mL
Triglyceride	5.0 mg/mL










## 17. PERFORMANCE

<b>Analytical Specificity (Cross Reactivity)</b>	No cross-reactivities were found to:		RSV, Adenovirus, Yersinia	
<b>Precision</b>	Mean (U/mL)	CV (%)		
	Intra-Assay	27	8.1	
	Inter-Assay	28	11.3	
<b>Linearity</b>	Range (U/mL)	Serial dilution up to	Range (%)	
	4.6 - 115	1/8	77 - 125	
<b>Recovery</b>	88 – 107 %	% Recovery after spiking (n = 3)		
<b>Method Comparison versus ELISA</b>	Rel. Sensitivity	> 95 %		
	Rel. Specificity	> 95 %		

**18. PRODUCT LITERATURE REFERENCES**

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# Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλισμένο
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazemar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbicante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>Symbols of the kit components see MATERIALS SUPPLIED.  Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.  Voir MATERIEL FOURNI pour les symbôles des composants du kit.  Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.  Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.  Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.  Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.</p>	

**COMPLAINTS:** Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

**WARRANTY:** The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

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