Helicobacter pylori

IgG – ELISA

Catalog number : NB-06-0488

Enzyme immunoassay for the quantitative determination of IgG-class antibodies against Helicobacter pylori in human serum or plasma

For laboratory use only.

1. INTRODUCTION

Helicobacter pylori is a spiral Gram-negative bacterium (2-6.5 µm in size, flagellated) which colonizes the human gastric mucosa. The organism is found in the mucous layer and adheres to the surface mucous epithelium of the stomach but generally does not penetrate the gastric mucosa directly.
However, there is a secondary inflammatory response in the mucosa leading to chronic active gastritis. Helicobacter pylori is the primary causative agent in most cases of peptic ulcer disease. Infection rate in Europe is about 30%-40%, worldwide about 50%. There is an inverse relationship between the presence of Helicobacter pylori infection and socioeconomic status. In developing countries, people acquire the infection at an early age such that by young adulthood as many as 90% of the population might have Helicobacter pylori gastritis. In developed western countries the prevalence of Helicobacter pylori gastritis is much lower. Under these conditions, the rate of acquisition is much slower (roughly 1% per annum) and the older one is, the more likely one is to be infected with the organism.

Species	Disease	Mechanism of infection
Helicobacter pylori	Gastritis Duodenal and peptic ulcers gastric cancer	The epidemiology of Helicobacter pylori suggests that transmission is via the oral route especially in areas with poor sanitation.

Infection may be identified by

- Histology: Giemsa, Warthin Starry or Genta stain / culture of antral biopsy specimens
- Enzymology: Detection of bacterial urease (urea breath test)
- Serology: Detection of antibodies

A positive antibody test implies active gastritis unless the patient has received a specific anti-H. pylori therapy previously. The advantage of serial ELISA testing is that, with prolonged follow up after therapy, patients can be classified as cured if their ELISA levels decrease significantly.

2. INTENDED USE

The Neo Biotech Helicobacter pylori IgG-ELISA is intended for the qualitative and **quantitative** determination of IgG class antibodies against Helicobacter pylori in human serum or plasma (citrate).

3. PRINCIPLE OF THE ASSAY

The quantitative immunoenzymatic determination of IgG-class antibodies against Helicobacter pylori is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiter strip wells are precoated with Helicobacter pylori antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human IgG conjugate is added. This conjugate binds to the captured Helicobacter specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of Helicobacter specific IgG antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1 Reagents supplied

- **Helicobacter pylori Coated Wells (IgG):** 12 breakapart 8-well snap-off strips coated with Helicobacter pylori antigen; in resealable aluminium foil.
- **IgG Sample Diluent** ***: 1 bottle containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2, coloured yellow; ready to use; white cap.
- **Stop Solution**: 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Solution (20x conc.)*:** 1 bottle containing 50 ml of a 20-fold concentrated buffer for washing the wells; pH 7.2 ± 0.2; white cap.
- **Helicobacter pylori anti-IgG conjugate**:** 1 bottle containing 20 ml of peroxidase labelled antibodies to human IgG; coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetra-methyl-benzidine (TMB); ready to use; yellow cap.
- **Helicobacter pylori IgG Standards***:** 4 vials, each containing 2ml; ready to use:

Standard A:	0	NTU/ml; blue cap
Standard B:	15	NTU/ml; green cap
Standard C:	75	NTU/ml; yellow cap
Standard D:	150	NTU/ml; red cap

* contains 0.1 % Bronidox L after dilution
** contains 0.2 % Bronidox L
*** contains 0.1 % Kathon

4.2. Materials supplied

- 1 Strip holder
- 1 Cover foil
- 1 Test protocol
- 1 distribution and identification plan

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (20…25°C) before starting the test run!

6.1. Coated snap-off Strips

The ready to use breakapart snap-off strips are coated with Helicobacter pylori antigen. Store at 2...8°C. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C; stability until expiry date.*

6.2. Helicobacter pylori anti-IgG Conjugate

The bottle contains 20 ml of a solution with anti-human IgG horseradish peroxidase, buffer, stabilizers, preservatives and an inert blue dye. The solution is ready to use. Store at 2...8°C. *After first opening until expiry date when stored at 2…8°C.*

6.3. Standards

The vials labelled with Standard A, B, C and D contain a ready to use standard solution. The concentration of the standards in Neo Biotech U nits (NTU) are:

Standard A: 0 NTU/ml
Standard B: 15 NTU/ml
Standard C: 75 NTU/ml
Standard D: 150 NTU/ml

The solutions have to be stored at 2...8°C and contain 0.1% Kathon. *After first opening until expiry date when stored at 2…8°C.*

6.4. IgG Sample Diluent

The bottle contains 100 ml phosphate buffer, stabilizers, preservatives and an inert yellow dye. It is used for the dilution of the patient specimen. This ready to use solution has to be stored at 2… 8°C. *After first opening until expiry date when stored at 2…8°C.*

6.5. Washing Solution (20xconc.)

The bottle contains 50 ml of a concentrated buffer, detergents and preservatives. Dilute washing solution 1+19; e.g. 10 ml washing solution + 190 ml fresh and germ free redistilled water. The diluted buffer will keep for 5 days if stored at room temperature. *Crystals in the solution disappear by warming up to 37 °C in a water bath. After first opening the concentrate is stable until the expiry date.*

6.6. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C, away from the light. *The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first opening until expiry date when stored at 2…8°C.*

6.7. Stop Solution

The bottle contains 15 ml 0.2 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2 … 8°C. After first opening stability until expiry date.

7. SPECIMEN COLLECTION AND PREPARATION

Use human serum or plasma (citrate) samples with this assay. If the assay is performed within 5 days after sample collection, the specimen should be kept at 2...8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. *Avoid repeated freezing and thawing.*
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10µl sample and 1ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300µl to 350µl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

1 well	(e.g. A1)	for the substrate blank,
4 wells	(e.g. B1, C1, etc.)	for Standard A, B, C and D.

It is recommended to determine standards and patient samples in duplicate.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each control and sample.

Adjust the incubator to 37° ± 1°C.

1. Dispense 100µl of each Standard (A, B, C and D) and diluted samples into the respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37±1°C.**
4. When incubation has been completed, remove the foil, aspirate the content off the wells and wash each well three times with 300µl of washing solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 100µl Helicobacter pylori anti-IgG Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature (20 to 25°C).** *Do not expose to direct sunlight.*
7. Repeat step 4.
8. Dispense 100µl TMB Substrate Solution into all wells
9. **Incubate for exactly 15 min at room temperature (20 to 25°C) in the dark.**
10. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
    Any blue colour developed during the incubation turns into yellow.

    Note: Highly positive patient samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample1+100 with dilution buffer and multiply the results in NTU by 2.
11. Measure the absorbance of the specimen at 450/620nm within 30 min after addition of the Stop Solution.

8.2. Measurement

Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and patient sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Assay validation criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank** in A1: Absorbance < **0.100.**
- **Standard A** in B1: Absorbance < **0.200**
  Standard B in C1: Absorbance > **0.200**
  Standard C in D1: Absorbance > **0.500**
- **Standard D** in E1: Absorbance > **1.100**

Standard A < Standard B < Standard C < Standard D

If these criteria are not met, the test is not valid and must be repeated.

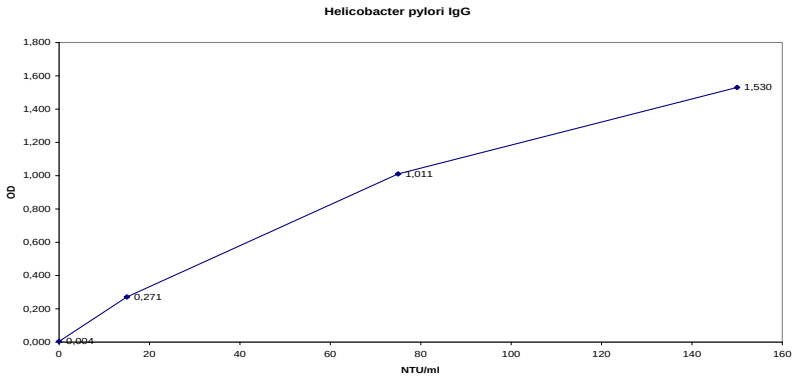
9.2. Calculation of Results

In order to obtain **quantitative results in NTU/ml** blot the (mean) absorbance values of the 4 Standards A, B, C and D on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0, 15, 75 and 150 NTU/ml) and draw a standard calibration curve (absorbance values on the vertical y-axis, concentrations on the horizontal x-axis).

Read results from this standard curve employing the (mean) absorbance values of each patient specimen and control.

All suitable computer programs available can be used for automated result reading and calculation.

9.3. Typical Calibration Curve



9.4. Interpretation of Results

Normal value ranges for this ELISA should be established by each laboratory based on its own patient populations in the geographical areas serviced.

The following values should be considered as a guideline:

Reactive **> 20 NTU/ml**

Grey zone (equivocal): **15 - 20 NTU/ml**

Non reactive: **< 15 NTU/ml**

10. SPECIFIC PERFORMANCE CHARACTERISTICS

10.1. Precision

Interassay	n	Mean	Cv (%)	Cv (%) mean
Pos Serum	13	1.3	7.2	5.1
	13	3.7	3.0	

Intraassay	n	Mean	Cv (%)	Cv (%) mean
Pos Serum	19	0.56	6.1	6.5
	19	1.50	7.0	

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 92.0% (95% confidence interval 0.84 – 0.99).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

It is 94.4 % (95% confidence interval 0.87 – 1).

10.4. Analytical Sensitivity

The analytical sensitivity is defined as the apparent concentration of the analyte that can be distinguished from the zero calibrator.
It is 3 NTU/ml.

10.5. Interferences

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

Note: The results refer to the groups of samples investigated; these are not guaranteed specifications.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.
In immunocompromized patients and newborns serological data only have restricted value.

12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- The Neo Biotech ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

WARNING:	In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!
WARNING:	Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: NB-06-0488 Helicobacter pylori IgG-ELISA (96 Determinations)

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Symbols Key	
	Manufactured by
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
	Expiration Date
	Storage Temperature
CE	CE Mark
[REF]	Catalogue Number
	Consult Instructions for Use
MTP	Microplate
CONJ	Conjugate
CAL	Calibrator
DIL G	Sample diluent buffer IgG
SOLN STOP	Stop solution
SUB TMB	TMB Substrate solution
WASHBUF 20x	Washing solution 20x concentrated
	Contains sufficient for “n” tests

SCHEME OF THE ASSAY

Helicobacter pylori IgG-ELISA

Assay Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and standards on the form supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate blank (z.B. A1)	Standard A	Standard B	Standard C	Standard D	Sample (diluted 1+100)
Standard A	-	100µl	-	-	-	-
Standard B	-	-	100µl	-	-	-
Standard C	-	-	-	100µl	-	-
Standard D	-	-	-	-	100µl	-
Sample (1+100 diluted)	-	-	-	-	-	100µl
Cover wells with foil supplied in the kit **Incubate for 1 h at 37°C** Wash each well three times with 300µl of washing solution						
Conjugate	-	100µl	100µl	100µl	100µl	100µl
Cover wells with foil supplied in the kit **Incubate for 30 min at room temperature** Wash each well three times with 300µl of washing solution						
TMB Substrate Solution	100µl	100µl	100µl	100µl	100µl	100µl
Incubate for15 min at room temperature in the dark						
Stop Solution	100µl	100µl	100µl	100µl	100µl	100µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)						

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