*recom*Line Helicobacter IgG 2.0
*recom*Line Helicobacter IgA 2.0

MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose

The *recom*Line Helicobacter IgG 2.0, IgA 2.0 is a qualitative test for the detection of IgG and IgA antibodies against *Helicobacter pylori* in human serum or plasma.

2 Intended use

The *recom*Line Helicobacter IgG 2.0, IgA 2.0 is a line immunoassay. In contrast to ELISA, the test principle enables the reliable identification of specific antibodies against selected antigens of *Helicobacter pylori* through the separate line-up of the individual antigens.
By using the type-specific antigen CagA, it is also possible to differentiate between an infection with type I and type II strains.
The *recom*Line Helicobacter IgG 2.0, IgA 2.0 can be used as a screening test or as a confirmatory test to clarify unclear screening results.

3 Test principle

Highly purified recombinant *Helicobacter pylori* antigens (CagA, VacA, GroEL, FliD, HpaA, gGT, HtrA, NapA, HP231 and CtkA) are fixed on nitrocellulose membrane strips.

1. The test strips are incubated with the diluted serum or plasma sample, and the specific antibodies bind to the pathogen antigens on the test strips.
2. Unbound antibodies are then flushed away.
3. In a second stage, the strips are incubated with anti-human immunoglobulin antibodies (IgG and IgA), which are coupled to horseradish peroxidase.
4. Unbound conjugate antibodies are then flushed away.
5. Specifically bound antibodies are detected with a staining reaction catalysed by the peroxidase. If an antigen-antibody reaction has taken place, a dark band will appear on the strip at the corresponding point.

There are control bands at the upper end of the test strips:

a) The reaction control band under the strip number, which must show a reaction for every serum/plasma sample.
b) The conjugate controls (IgG, IgA) are used to check the detected antibody class. If the test strip is used to detect IgG antibodies, the IgG conjugate control takes the form of a clearly coloured band. When IgA is detected, a clearly coloured IgA conjugate control band will appear.
c) "Cut-off control": The intensity of this band allows the assessment of the reactivity of each antigen band (see 9.2. Evaluation).

4 Reagents

4.1 Package contents

The reagents in one package are sufficient for 20 tests.

Each test kit contains:

WASHBUF A 10 X	**100 ml** Wash Buffer A (**10 times concentration**) Contains phosphate buffer, NaCl, KCl, detergent, preservative: MIT (0.1%) and oxypyrion (0.2%)
SUBS TMB	**40 ml** tetramethylbenzidine chromogenic substrate (**TMB, ready to use**)
MILKPOW	**5 g** skimmed milk powder
INSTRU	**1** instructions for use
EVALFORM	**1** Evaluation form

4.1.1 *recom*Line Helicobacter IgG 2.0
In addition to the components listed in 4.1, each test kit contains:

TESTSTR	**2** tubes, each with 10 numbered test strips
CONJ IgG	**500 µl** anti-human IgG conjugate **(100-fold concentration, green cap)** obtained from rabbits, contains NaN3 (<0.1%), MIT (<0.1%) and chlorazetamide (<0.1%)

4.1.2 *recom*Line Helicobacter IgA 2.0
In addition to the components listed in 4.1, each test kit contains:

TESTSTR	**2** tubes, each with 10 numbered test strips
CONJ IgA	**500 µl** anti-human IgA conjugate **(100-fold concentration, colourless cap)** obtained from rabbits, contains NaN3 (<0.1%), MIT (<0.1%) and chlorazetamide (<0.1%)

4.2 Materials required but not supplied

- Incubation trays (can be purchased as needed from MIKROGEN)
- Deionised water (high quality)
- Plastic forceps
- Horizontal shaker
- Vortex mixer or other rotators
- Vacuum pump or similar device
- Volumetric cylinders, 50 ml and 1000 ml
- Micropipettes with disposable tips, 20 µl and 1000 µl
- 10 ml pipette or dispenser
- Timer
- Absorbent paper towels
- Disposable protective gloves
- Waste container for bio-hazardous materials

5 Shelf life and handling

- Store reagents at +2 to +8°C before and after use, **do not freeze**.
- Subject all ingredients to room temperature (+18 to +25 °C) for at least 30 minutes before beginning the test. The test procedure is carried out at room temperature.
- Wash buffer, milk powder, dilution buffer, conjugates and TMB can be exchanged between different *recom*Line and/or *recom*Blot test systems if these components have the same symbol. Special attention should be paid to the expiry dates of these components.
- Mix the concentrated reagents and patient serums thoroughly before use. Avoid a build up of foam.
- Only open the tube containing the test strip immediately before use to avoid condensation. Strips that are not required remain in the tube and continue to be stored at +2°C to +8°C (reseal tubes well, test strips must not be exposed to humidity before starting the test!).
- The strips are marked with the serial number, as well as the test code.
- The packages bear an expiration date. After this has been reached, no guarantee of quality can be offered.
- Protect kit components from direct sunlight throughout the entire test procedure. The substrate solution (TMB) is especially sensitive to light.
- The test should only be carried out by trained and authorised personnel.
- In the event of substantial changes to the product or the regulations concerning use by the user, the application may not fulfil the purpose as specified by MIKROGEN.
- Cross-contamination of patient samples or conjugates can lead to inaccurate test results. Carefully add patient samples, test strips and the conjugate solution. Make sure that incubation solutions do not flow over into other wells. Carefully remove liquids.
- The strips must be completely wetted and submerged throughout the entire procedure.
- Automation is possible; further information can be obtained from MIKROGEN.

6 Warnings and precautions

- For *In vitro* diagnostic use only.
- All blood products must be treated as potentially infectious.
- The test strips were produced with inactivated whole-cell lysates and/or recombinant bacterial, viral or parasite antigens.
- After adding the patient or control material, the strips must be regarded as potentially infectious and be treated correspondingly.
- Suitable disposable gloves must be worn throughout the entire test procedure.
- The reagents contain the antimicrobial agents and preservatives sodium azide (NaN3), MIT (methylisothiazolinone), oxypyrion, chloroacetamide and hydrogen peroxide. Avoid contact with the skin or mucous membrane. Sodium azide (NaN3) can form an explosive azide upon contact with heavy metals such as copper and lead.
- All siphoned liquids must be collected. All collective containers must contain suitable disinfectants for the inactivation of human pathogens and be autoclaved. All reagents and materials contaminated with potentially infectious samples must be treated with ap-

propriate disinfectants or disposed of according to your hygiene regulations. The concentrations and incubation periods of the manufacturer must be observed.
- Use incubation trays only once.
- Handle strips carefully using plastic forceps.
- Do not substitute or mix the reagents with reagents from other manufacturers.
- Read through the entire instructions for use before carrying out the test and follow them carefully. Deviation from the test protocol provided in the instructions for use can lead to erroneous results.

7 Sampling and preparation of reagents

7.1 Samples

The sample can be serum or plasma (citrate, EDTA, heparin, CPD), which needs to be separated from the blood clot as soon as possible after sampling so as to avoid haemolysis. Avoid microbial contamination of the samples. Insoluble substances must be removed from the sample before incubation.
The use of heat-inactivated, icteric, haemolytic, lipaemic or turbid samples is not recommended.

Caution!
If analysis cannot take place immediately, the sample material may be stored for up to 2 weeks at +2°C- +8°C. Extended storage of the samples is possible at -20 °C or below. Repeated freezing and thawing of samples is not recommended due to the risk of inaccurate results. More than 3 freezing and thawing cycles should be avoided.

7.2 Preparation of solutions

7.2.1 *Preparation of ready-to-use wash buffer A*
This buffer is required for serum and conjugate dilution as well as washing stages.
The volume of wash buffer A for the corresponding number of tests must be determined before dilution.
The skimmed milk powder is first dissolved in washing buffer A-concentrate, after which this mixture is then topped up to the final volume with deionised water (dilution 1 + 9). The quantities required for a defined number of test strips are to be mathematically determined according to the following formulas (device-specific dead volume is not considered):

Reagent	Formula	Example: 5 strips
Skimmed milk powder [g]	= number of strips x 0.1	0.5 g
Wash buffer A concentrate [ml]	= number of strips x 2	10 ml
Deionised water [ml]	= number of strips x 18	90 ml
Ready-to-use wash buffer A [ml]	= number of strips x 20	100 ml

Ready to use wash buffer A can be stored at 2 °C – +8°C for up to four weeks. The ready-to-use wash buffer A is odourless and slightly turbid.

7.2.2 *Preparation of conjugate solutions*
The conjugate solution must be prepared immediately before use. It is not possible to store the ready-to-use conjugate solution.
One part of the conjugate concentrate is diluted with 100 parts of the ready-to-use wash buffer A (1 + 100).
The quantities required for a defined number of test strips are to be calculated according to the following formula:

Reagent	Formula	Example: 5 strips
Conjugate concentrate [µl]	= number of strips x 20	100 µl
Ready-to-use wash buffer A [ml]	= number of strips x 2	10 ml

The conjugate quantities are calculated without dead volume. Depending on handling (manual or on a device), please mix additional conjugate solution for 1 to 3 strips.

8 Test procedure

No.	Execution	Note
1	Subject all reagents to 18 to +25°C (room temperature) for at least 30 minutes before beginning the test.	The test procedure is carried out at room temperature.
2	Preparing test strips Place the strips in **2 ml** of ready-to-use **wash buffer A**.	Do not touch the strips with bare hands - use the forceps. The strip number points upward. Place each strip in a separate well in the incubation tray (see 4.2). The strips must be completely immersed.
3 a) b)	Incubation of samples **20 µl** of undiluted sample (human serum or plasma) is pipetted on to the test strip for each incubation mixture (Dilution 1 + 100) Incubate for **1 hour** with gentle shaking	 Pipette the sample at one end of the immersed strip in the wash buffer A and mix as quickly as possible by carefully shaking the tray. Cover the incubation tray with plastic cover and place in the shaker.
4 a) b) c)	Washing Carefully remove the plastic cover from the incubation trays. Gently siphon serum dilution from the individual wells. Pipette **2 ml** of ready to use **wash buffer A** into each well, wash for 5 minutes with gentle shaking and then siphon off the wash buffer A.	Washing steps 8.4a-8.4c are to be carried out a total of three times. Avoid cross-contamination. The manufacturer's instructions must be observed during automatic processing.
5	Incubation with conjugate Add **2 ml** of ready-to-use **conjugate solution** and incubate for **45 minutes** while shaking gently.	Cover the incubation tray with plastic cover and place in the shaker.
6	Washing see 8.4	Carry out the washing stages three times in total (see 8.4a-8.4c)
7	Substrate reaction Add **1.5 ml** of **substrate solution** and incubate for **8 minutes** while shaking gently.	
8	Stopping the reaction Wash at least three times **briefly** with **deionised water**.	
9	Drying the strips Dry strip between 2 layers of absorbent paper for **2 hours** before analysis.	Carefully remove strips from the water using plastic forceps. Store strips away from light.
Caution! **Incubation solutions must not flow into other wells. Splashing must be avoided especially when opening and closing the lid.**		

9 Results

Caution:
Please do not use automated interpretation without considering the information on interpretation given below.

9.1 Validation – Quality Control

An analysis of the test can be carried out if the following criteria have been fulfilled:
1. Reaction control band (top line) with clearly visible colour, dark band.
2. Antibody class (second and third band): the IgG or IgA conjugate control band must have a distinct colour. The respective other conjugate band may show weak colouring.
3. Cut-off control (fourth band): weak yet visible colour.

9.2 Evaluation

The evaluation of the test strips can be visual or computer-assisted - using the test strip analysis software *recom* Scan. The *recom*Scan software is designed to support the evaluation of test strips. Further information and related instructions for the computer-assisted evaluation is available on request from MIKROGEN. The following instructions relate to visual analysis.

9.2.1 *Assessment of band intensity*
1. Note the date, batch and tube number as well as the detected antibody class on the attached evaluation sheet.
2. Enter the sample identification numbers in the evaluation form.
3. Now stick the corresponding test strips on to the appropriate fields on the evaluation form using a glue stick. To do this, align the test strips with the reaction control bands along the marking line indi-

cated. Then use transparent adhesive tape to stick the test strips down to the left of the marking line (do not put tape over the reaction control bands!). Sticking the entire test strip down flat using glue or tape can lead to changes in colour.

4. Now identify the bands of the developed test strip using the control strip printed on the evaluation form and enter them in the evaluation form. For each corresponding immunoglobulin class, assess separately the intensity of the bands occurring on the basis of Table 1.

Table 1: Assessment of band intensity in relation to the cut-off band

Stain intensity of the bands	Assessment
No reaction	-
Very low intensity (lower than cut-off band)	+/-
Low intensity (equivalent to cut-off band)	+
Strong intensity (stronger than cut-off band)	++
Very strong intensity	+++

+, ++ and +++ are to be rated positive (p); - and +/- are to be rated negative (n)

9.3 Interpretation of test results

The test result is determined by adding the point values according to Table 2 the various reactive ≥ cut-off bands (i.e. using bands rated at least as +). The resulting sum is entered in the column with the sum symbol.
The positive, inconclusive or negative assessment of the sample can then be determined directly with the Table 3 and entered in the assessment column of the evaluation sheet.

Table 2: Point evaluation of the antigens (IgG and IgA)

Antigen	Points
CagA	4
VacA	4
GroEL	2
FliD	2
HpaA	2
gGT	2
HtrA	2
NapA	1
HP231	1
CtkA	1

Table 3: Test interpretation (IgG and IgA)

Sum of points	Assessment
≤ 2	negative
3	inconclusive
≥ 4	positive

The type differentiation is carried out using the CagA antigen and is only possible in the IgG. If the CagA bands react above the cut-off (i.e. with at least +), there is an infection of *Helicobacter pylori* Type I present.

10 Limitations of the method - restrictions

- Serological test results must always be considered in the context of other medical assessments of the patient. Therapeutic consequences of the serological findings must always be taken in context with the clinical data.
- A negative test result for *Helicobacter pylori* cannot exclude an infection with *Helicobacter pylori*. If there is persistent clinical suspicion of an infection with *Helicobacter pylori*, further diagnostic tests such as histological examinations should be carried out.
- A positive result in *recom*Line Helicobacter IgG and/or IgA 2.0 does not always mean that an active disease is present.
- The two most important virulence markers of *H. pylori* are CagA (cytotoxin-associated gene A) and VacA (vacuolating cytotoxin A). Infections with CagA-positive *H. pylori* strains (type I strains) are associated with a much higher risk of pre-malignant changes / gastric carcinoma, MALT lymphoma and ulcer. Unlike CagA-negative strains, these strains also generally exhibit a cytotoxic variant of VacA.
- Dark test strips: Some patient samples may generate a dark, consistent or mottled colouring of the entire nitrocellulose strip (e.g. the serums of patients with lactic protein allergies). Various factors in each patient serum are responsible for this. The evaluation of these strips is usually only partly feasible. Thus, "inverse" bands (white bands on dark background), for example, should be evaluated as negative. The respective serum should always be examined using other serological methods.

11 Test performance

11.1 Diagnostic sensitivity

	Histology/culture positive samples (n = 139)	
*recom*Line Helicobacter 2.0	IgG	IgA
Negative	1	51
Inconclusive	0	0
Positive	138	88
Diagnostic sensitivity	**99.3%**	**63.3%**

11.2 Diagnostic specificity

	Histology/culture negative samples (n = 97)	
*recom*Line Helicobacter 2.0	IgG	IgA
Negative	97	97
Inconclusive	0	0
Positive	0	0
Diagnostic specificity	**100%**	**100%**

11.3 Prevalence

	Blood donations (n=100)	
*recom*Line Helicobacter 2.0	IgG	IgA
Negative	75	89
Inconclusive	0	0
Positive	25	11
Prevalence	25%	11%

11.4 Analytical specificity

The analytical specificity is defined as the capacity of the test to precisely determine the analytes in the presence of potential interference factors in the sample matrix (e.g. anticoagulants, haemolysis, effects of the sample handling) or cross reactions with potentially interfering antibodies.

a) Interferences: Control studies on potentially interfering factors have shown that anticoagulants (sodium citrate, EDTA, heparin), haemolysis, lipaemia or bilirubinaemia or cycles of freezing and thawing do not affect the performance of the test.

b) Cross-reactions: The potential interference of antibodies with other related organisms (*Campylobacter jejuni*) has been investigated in control studies. Also tested were conditions caused by an atypical activity of the immune system, such as *rheumatoid factor*. No cross-reactivities were detected.

12 Literature

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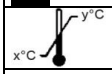
We will gladly send you further literature on the diagnosis of Helicobacter upon request.

13 Explanation of symbols

	Content is sufficient for <n> applications Number of applications
WASHBUF A 10 X	Wash buffer A (ten-fold concentration)
SUBS TMB	Tetramethylbenzidine chromogenic substance
MILKPOW	Skimmed milk powder
EVALFORM	Evaluation form
INSTRU	Instructions for use
TESTSTR	Test strips
CONJ IgG	Anti-human IgG conjugate
CONJ IgA	Anti-human IgA conjugate
	See instructions for use
CONT	Content, includes
IVD	In vitro diagnostic test
LOT	Batch/version number
	Do not freeze
REF	Order number
	Best before Expiry date
	Store at x°C to y°C
	Manufacturer

14 Manufacturer and version information

***recom*Line Helicobacter IgG 2.0** ***recom*Line Helicobacter IgA 2.0**		Item No. **4774** Item No. **4775**
Instructions for use valid from		GARLHP005EN 2023-05
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