

# RIDASCREEN<sup>®</sup> Helicobacter IgA, IgG

Article no: K2311 (IgA)  
K2321 (IgG)



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## 1. Intended use

For *in vitro* diagnostic use. The RIDASCREEN® Helicobacter tests are enzyme immunoassays for the semi-quantitative determination of IgA or IgG antibodies against Helicobacter pylori in human serum. The tests should be used for confirmation purposes when there is a suspected case of infection with helicobacter or for clarifying the immune status.

## 2. Summary and explanation of the test

After infection with helicobacter, specific antibodies are formed against the pathogen as a result of the response from the immune system. By using immunological methods, it is possible to determine the antibodies in the serum. The test method used and the choice of the pathogen-specific antigen both have a significant bearing on the meaningfulness of the test. Because it is possible to differentiate between the individual immunoglobulin classes in the enzyme immunoassay, more precise statements about the immunological status of a patient can be made than those which are based on other serological methods (such as the haemagglutination inhibition test or the complement fixation reaction test).

## 3. Test principle

Purified antigens are coated to a microwell plate. Antibodies in the patient samples bind to the antigens and are determined during the second incubation step by using enzyme-labelled anti-human antibodies (the conjugate). The enzyme converts the colourless substrate (H<sub>2</sub>O<sub>2</sub>/TMB) to a blue end product. The enzyme reaction is stopped by adding sulphuric acid and the colour of the mixture switches from blue to yellow at the same time. The final measurement is carried out at 450 nm on a photometer using a reference wavelength  $\geq 620$  nm.

## 4. Reagents provided

Table 1: Pack contents (there are enough reagents in a pack for 96 determinations)

			K2311 IgA	K2321 IgG
<b>Plate</b>	96 det.	Microwell plate; 12 microwell strips (can be divided) in the strip holder; coated with the H. pylori antigen	X	X
<b>SeroPP</b>	110 ml	Sample buffer, ready for use; phosphate-buffered NaCl solution; coloured yellow; contains 0.01% thimerosal and 0.05% tween 20	X	X
<b>SeroWP</b>	100 ml	Wash buffer, 10-fold concentrate; tris-buffered NaCl solution; contains 0.2% bronidox-L and 0.5% tween 20	X	X
<b>Control IgA</b> <input type="checkbox"/> + <i>blue lid</i>	2.5 ml	Standard control IgA, ready for use; diluted human serum; coloured blue; contains 0.01% thimerosal and 0.05% tween 20	X	
<b>Control IgG</b> <input type="checkbox"/> + <i>green lid</i>	2.5 ml	Standard control IgG, ready for use; diluted human serum; coloured green; contains 0.01% thimerosal and 0.05% tween 20		X
<b>Control IgA</b> <input type="checkbox"/> - <i>colourless lid</i>	1.2 ml	Negative control IgA, ready for use; diluted human serum; contains 0.01% thimerosal and 0.05% tween 20	X	
<b>Control IgG</b> <input type="checkbox"/> - <i>colourless lid</i>	1.2 ml	Negative control IgG, ready for use; diluted human serum; contains 0.01% thimerosal and 0.05% tween 20		X
<b>SeroA LD</b> <i>blue lid</i>	12 ml	Anti-human IgA conjugate (goat), ready for use; peroxidase conjugated antibodies in stabilised protein solution; contains 10 ppm proclin, 0.01% methylisothiazolinone, 0.01% bromonitrodioxane	X	
<b>SeroG HD</b> <i>green lid</i>	12 ml	Anti-human IgG conjugate (rabbit); ready for use; peroxidase conjugated antibodies in stabilised protein solution; contains 10 ppm proclin, 0.01% methylisothiazolinone, 0.01% bromonitrodioxane		X
<b>SeroSC</b>	12 ml	Substrate; H <sub>2</sub> O <sub>2</sub> /tetramethylbenzidine; ready for use	X	X
<b>SeroStop</b>	12 ml	Stop reagent 0.5 M sulphuric acid; ready for use	X	X

## 5. Storage instructions

The test kit must be stored at 2 – 8 °C and can be used until the expiry date printed on the label. The diluted wash buffer can be used for a maximum of 4 weeks when stored at 2 – 8 °C or for 5 days when stored at room temperature (20 – 25 °C). After the expiry date, the quality guarantee is no longer valid.

The aluminium bag containing the microwell plate must be opened in such a way that the clip seal is not torn off. Any microwell strips which are not required must be immediately returned to the aluminium bag and stored at 2 – 8 °C.

The reagents also must not be allowed to become contaminated and the colourless substrate must be protected from exposure to direct light.

## 6. Additional necessary reagents – and necessary equipment

### 6.1. Reagents

- distilled or deionised water

### 6.2. Accessories

- Moist chamber / incubator at 37 °C
- Test tubes
- Vortex mixer
- Micropipettes for 10-100 µl and 100-1000 µl capacities
- Measuring cylinder (1000 ml)
- Stop clock
- Microplate washer or multichannel pipette
- Microplate reader (450 nm, reference wavelength ≥ 620 nm)
- Filter paper (laboratory towels)
- Waste container containing 0.5 % sodium hypochlorite solution

## 7. Precautions for users

For *in vitro* diagnostic use only.

This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories must be followed and the instructions for carrying out the test must be strictly adhered to.

Samples or reagents must not be pipetted by mouth and contact with injured skin or mucous membranes must be prevented. When handling the samples, wear disposable gloves and when the test is finished, wash your hands. Do not smoke, eat or drink in areas where samples or test reagents are being used.

The control sera (standard control and negative control) in the kit have been tested for HIV- and HCV-Ab as well as HbsAg with negative results. Nevertheless, they must be treated as potentially infectious in the same way as the patient samples and all other materials with which they come into contact and they must be handled in accordance with the relevant national safety regulations.

The standard control and negative control as well as the sample buffer contain 0.01 % thimerosal as a preservative. This substance must not be allowed to come into contact with the skin or mucous membrane.

The wash buffer contains 0.2 % Bronidox-L as a preservative. This substance must not be allowed to come into contact with the skin or mucous membrane.

H<sub>2</sub>O<sub>2</sub> (substrate) can cause burns. Handle with care.

The stop reagent contains 0.5 M sulphuric acid. Avoid contact with the skin and clothing. If the skin is contaminated with the stop reagent, rinse it off with water.

All reagents and materials coming into contact with potentially infectious samples must be treated with suitable disinfectants or autoclaved at 121 °C for at least 1 hour. CAUTION: To prevent the formation of poisonous gases, any liquid waste containing stop reagent must be neutralized before adding it to hypochlorite solution.

## 8. Specimen collection and storage

The test has been developed for testing human serum samples. After blood collection, the blood should be separated from blood clots as soon as possible in order to prevent haemolysis. The samples must be stored cold or frozen until they are tested. Repeated freezing and thawing of the samples and microbial contamination must be prevented at all costs. Using heat-inactivated, lipaemic, haemolytic, icteric or turbid samples can lead to false results.

Table 2: Sample storage

Undiluted serum		Diluted serum
2 – 8 °C	-20 °C	2 – 8 °C
1 week	>1 week	7 hours

## 9. Test procedure

### 9.1. General information

All reagents and the microwell plate must be brought to room temperature (20 – 25 °C) before use. The microwell strips must not be removed from the aluminium bag until they have reached room temperature. The reagents must be thoroughly mixed immediately before use. After use, the kit must be immediately returned to storage at 2 – 8 °C.

Take only the volume of reagents that is needed for test procedure. Do not pour reagents back into vials as reagent contamination may occur. Do not pour reagents back into vials as this may lead to reagent contamination.

The microwell strips cannot be used more than once. The reagents and microwell strips must not be used if the packaging is damaged or the vials are leaking.

Some of the reagents in the kit are not test specific. The reagents labelled Sero (such as **SeroPP**) can also be used with other RIDASCREEN® Sero EIAs with the corresponding reagents.

**The control sera relate to the lot. Control sera from kits with different lot numbers must not be exchanged.**

### 9.2. Preparing the wash buffer

1 part wash buffer concentrate SeroWP is mixed with 9 parts distilled water. In order to do this, place 100 ml of the concentrate in a 1000 ml measuring cylinder and make up the solution to 1000 ml with distilled water. Any crystals present in the concentrate must be dissolved beforehand by warming in a water bath at 37 °C. The diluted wash buffer can be used for a maximum of 4 weeks when stored at 2 – 8 °C or for 5 days when stored at room temperature (20 – 25 °C).

### 9.3. Preparing the samples

Dilute the serum samples to be tested with sample buffer **SeroPP** before starting the test 1:50.

e.g. 10 µl serum +490 µl **SeroPP**

#### **Note:**

**The negative control and standard control are ready for use and must NOT be diluted or absorbed.**

#### 9.4. First incubation

After insertion of a sufficient number of wells into the frame, pipette 100 µl diluted sera and 100 µl ready-to-use control into each of the corresponding wells leaving Position A1 (reagent blank value) empty. Add the negative control **Control IgA -** or **Control IgG -** once and the standard control **Control IgA +** or **Control IgG +** in duplicate. Cover the plate and incubate at 37 °C for 30 minutes in a moist chamber / incubator. During this process, the bottoms of the wells must not be in contact with heat-conductive materials (such as metals or moist paper). The microwell plate must be covered during incubation.

The controls which correspond to the determination (IgA or IgG) are to be used.

A1	Reagent blank value
B1	Negative control
C1	Standard control
D1	Standard control
E1, F1	Patient serum 1 and 2 etc.

#### **Note:**

**The microwell plate must not be placed in a cold incubation container reaching 37 °C during incubation. The temperature of the container must be adjusted to 37 °C beforehand.**

#### 9.5. Washing

The wells must be emptied into a waste container containing hypochlorite solution for disinfection. Then knock out the plate onto absorbent paper in order to remove the residual moisture. After this, wash the plate 4 times using 300 µl wash buffer each time. Make sure that the wells are emptied completely by knocking them out on an unused part of the absorbent paper after each wash.

**When using a microplate washer, make sure that the machine is correctly adjusted to the type of plate being used. After washing, knock out the plate onto clean absorbent paper in order to remove any residual moisture.**

#### 9.6. Second incubation

Add 100 µl conjugate **SeroA LD** or **SeroG HD** to the corresponding wells (including A1). Next, cover the plate and incubate at 37 °C for 30 minutes in a moist chamber / incubator (see Section 9.4).

## 9.7. Washing

Wash 4 times in accordance with Section 9.5.

## 9.8. Third incubation

Add 100 µl substrate **SeroSC** to each well. Then, cover the plate and incubate at 37 °C for 30 minutes in a moist chamber / incubator. After this, stop the reaction by adding 100 µl stop reagent **SeroStop** to each well. After mixing carefully (by lightly tapping the side of the plate), measure the absorbance at 450 nm (reference wavelength  $\geq$  620 nm) in a plate photometer. Calibrate the reagent blank value (Position A1) to zero.

### **Note:**

**The underside of the microwell plate must be wiped off to remove condensation water before the measurement is carried out.**

## **10. Quality control – indications of instability or deterioration**

For quality control purposes, the standard control (in duplicate) and the negative control must be used every time the test is carried out. The test has been carried out correctly if the average absorbance for the standard control at 450/620 nm is within the range stated on the enclosed data sheet. If the two individual measurements deviate from the average by more than 20 %, the test must be repeated. The absorbance for the negative control at 450/620 nm must be  $< 0.3$ .

If the values differ from those required, if the reagent is turbid or the substrate has turned blue before adding to the wells, it may indicate that the reagents have expired.

If the stipulated values are not met, the following points must be checked before repeating the test:

- Expiry date of the reagents used
- Functionality of the equipment being used (e.g. calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks – a substrate solution which has turned blue must not be used.

If the conditions are still not fulfilled after repeating the test, please contact your local R-Biopharm distributor.



## 11. Evaluation and interpretation

The test can be evaluated using three different methods:

1. By using the standard curve provided in the kit
2. By using the table of values (see data sheet provided in the kit)
3. Mathematically using the 4-parameter method or the  $\alpha$  method

**The reagent blank value must be subtracted from each measured value before the evaluation.**

### 11.1. Evaluation by using the standard curve provided in the kit

In order to carry out the evaluation using the standard curve, a correction must be made to the average value for the standard control first in order to take into account any fluctuations which may occur from one day to the next. The correction factor F is calculated from the current measured average value for the standard control and its target value. The target value, which depends on the lot, is recorded on the enclosed data sheet.

$$F = \frac{\text{standard control target value}}{\text{standard control measured average value}}$$

All OD values for the samples must be multiplied by the factor F. The corresponding U/ml values are then read off the standard curve using the corrected values.

### 11.2. Evaluation by using the table of values

	U/ml	Range of values for the standard control	
			0.89 - 0.94
-	< 10.0		< 0.17
?	10.0 - 16.0		0.17 - 0.26
+	16.1 - 30.0		0.27 - 0.47
	30.1 - 60.0		0.48 - 0.80
	60.1 - 110.0		0.81 - 1.27
	110.1 - 210.0		1.28 - 1.84
	210.1 - 400.0		1.85 - 2.39
	> 400.0		> 2.39

Figure 1: Example of an IgG determination

(Extract from a lot-specific data sheet)

The absorbance for the standard control is used to identify the column in the table with the range of values which applies to the current measurement. The measured absorbance for the sample is assigned to the appropriate range of values and then the titer in U/ml is read from the second column to the left in the table.

For example, the absorbance for the standard control for a certain measurement is 0.91. In this case, the column in the table with the range 0.89 - 0.94 is the one to use to determine the results. A patient sample with an absorbance of 0.61 therefore corresponds to the titer range 30.1 - 60.0 U/ml. (The values cited are merely to be regarded as examples and may differ from the current values on the data sheet.)

The evaluation for the determined results (positive (+), negative (-) or equivocal (?)) must be taken from the first column of the table of values.

### 11.3. Mathematical evaluation

The required values for mathematical evaluation according to the 4-parameter method or the  $\alpha$ -method are recorded on the enclosed data sheet.

### 11.4. Test result

Table 3: Evaluation of the determined units

	IgA	IgG
negative	< 10 U/ml	< 10 U/ml
equivocal	10 - 13 U/ml	10 - 16 U/ml
positive	> 13 U/ml	> 16 U/ml

## 12. Limitations of the method

RIDASCREEN® Helicobacter EIA detects IgA or IgG antibodies against H. pylori. The test cannot be used to derive a relationship between the extinction determined and occurrence of serious clinical symptoms. The results obtained must always be interpreted in combination with the clinical picture.

A negative result in the EIA cannot rule out infection with Helicobacter pylori. When infection with Helicobacter pylori is suspected for clinical reasons and the serological findings are negative, further sampling and testing should be carried out after four weeks.

An equivocal result could indicate the start of an immune response. Antibodies against *Helicobacter pylori* are present. Progressive seroconversion, which indicates an acute development of the disease, must be clarified by repeated testing after two to four weeks. An uncertain result may also be observed after therapy and recovery after infection.

Two consecutive samples of sera should always be collected from a patient and subjected to serological testing in order to improve the quality of the diagnostics. The progress of the titre is important for interpreting the findings.

A positive result in the EIA does not always mean that the disease is actively developing. Antibodies can also be determined during *Helicobacter pylori* colonisation without clinical manifestations. We recommend carrying out the RIDA<sup>®</sup>BLOT *Helicobacter* test in order to confirm the EIA result and identify highly virulent *Helicobacter pylori* strains by determining the antibodies against the pathogenicity factors CagA and VacA.

Only a few tests currently exist for the significance of IgA antibodies for an infection with *Helicobacter pylori*. We recommend carrying out this test when the IgG findings are unclear.

A positive result does not rule out the presence of another infectious pathogen as the cause of the disease.

### 13. Performance characteristics

Table 4: Inter-assay variation (n = 5)

<u>Inter-assay variation</u>	<u>IgA</u>		<u>IgG</u>	
	OD	CV	OD	CV
Serum 1	0.208	5.1 %	0.178	9.9 %
Serum 2	0.716	2.6 %	0.736	6.2 %
Serum 3	1.590	1.0 %	1.284	4.4 %

Table 5: Intra-assay variation (n = 24)

<u>Intra-assay variation</u>	<u>IgA</u>		<u>IgG</u>	
	OD	CV	OD	CV
Serum 1	0.127	5.8 %	0.119	3.3 %
Serum 2	0.691	3.0 %	0.536	4.8 %
Serum 3	2.154	1.8 %	1.164	3.0 %

Table 6: Sensitivity and specificity in comparison with two other commercial ELISAs

	IgA	IgG
Sensitivity	100.0 %	100.0 %
Specificity	98.0 %	100.0 %

Table 7: Results from testing 212 blood-donor sera taken from a blood donor center in Germany

212 blood donor sera	IgA	IgG
negative	63.7 %	64.1 %
equivocal	8.5 %	5.2 %
positive	27.8 %	30.7 %

## References

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