

# RIDASCREEN<sup>®</sup> Giardia

Article no.: C1101



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## 1. Intended use

For *in vitro* diagnostic use. RIDASCREEN® Giardia is an enzyme immunoassay for the qualitative identification of *Giardia lamblia* in human stool samples.

## 2. Summary and explanation of the test

*Giardia lamblia* are flagellate parasites that cause infections in the small intestines of humans after the oral uptake of giardia cysts. Because it is found worldwide, the *Giardia lamblia* pathogen is a significant cause of chronic diarrhea, in particular in medical cases related to travel. The infection occurs after ingestion of the cysts in contaminated food or water. In the USA, the *Giardia lamblia* pathogen is the most frequent cause of illness with diarrhea in connection with the intake of water. In underdeveloped countries, giardia infection is one of the most frequent causes of disease in children under the age of ten years, with a prevalence of 15–20%. Giardiasis (lambliasis) occurs as acute or chronic diarrhea, whereby asymptomatic persons can also excrete the cysts of the parasite. The incubation period ranges from 3 to 42 days.

The acute infection presents the following symptoms: sudden occurrence of watery diarrhea (frequent stools of yellow color, foamy consistence, and unpleasant smell, along with flatulence), loss of appetite, nausea, lethargy, and weight loss.

In the past, the method used most often to diagnose lambliasis was to examine stool samples under the microscope to detect the cysts, and this requires the availability of experienced staff. It is also necessary to conduct these tests in a sequence of multiple stool samples throughout a lengthy period of time, because the frequency of cyst excretion can vary largely.

## 3. Test principle

The RIDASCREEN® Giardia Test employs specific antibodies in a sandwich-type method. Giardia specific antibodies to the specific antigens of *Giardia lamblia* cysts and trophozoites are attached to the well surface of the microwell plate.

A pipette is used to place a suspension of the stool sample to be examined as well as control specimens into the well of the microwell plate together with biotinylated anti-giardia antibodies (Conjugate 1) for incubation at room temperature (20–25 °C). After a wash step, streptavidin poly-peroxidase conjugate (Conjugate 2) is added and it is incubated again at room temperature (20–25 °C). With the presence of the *Giardia lamblia* antigen in a specimen, the immobilized antibody, the *Giardia lamblia* antigen, and the conjugated antibody form a sandwich complex. Another wash step removes the unattached streptavidin poly-peroxidase conjugate. After adding the substrate, the attached enzyme changes the colour of the previously colourless solution in the wells of the microwell plate to blue if the test is positive.. Addition of a stop reagent changes the color from blue to yellow. The extinction is proportional to the concentration of *Giardia lamblia* antigens in the specimen.

#### 4. Reagents provided

The reagents in the kit are sufficient for 96 determinations.

Plate	96	Microwell plate, 12 microwell strips (which can be divided) in the strip holder; coated with specific monoclonal antibodies to the <i>Giardia lamblia</i> specific antigens.
Diluent   1	100 ml	Sample dilution buffer, protein-buffered NaCl solution, ready to use, blue colored
Wash	100 ml	Wash buffer, phosphate-buffered NaCl solution (concentrated 10-fold); contains 0.1 % thimerosal
Control   +	2 ml	Inactivated <i>Giardia lamblia</i> antigen; ready for use
Control   -	2 ml	Negative control (sample dilution buffer); ready for use
Conjugate   1	13 ml	Biotin-conjugated monoclonal antibodies to the <i>Giardia lamblia</i> specific antigens in stabilized protein solution; ready for use, red color Streptavidin poly-peroxidase conjugate in stabilized protein solution;
Conjugate   2	13 ml	ready for use; orange colored
Substrate	13 ml	Hydrogen peroxide/TMB; ready for use
Stop	12 ml	Stop reagent; 1 N sulphuric acid; ready for use

#### 5. Reagents and their storage

All reagents must be stored at 2–8 °C and can be used until the date printed on the label. Providing the diluted wash buffer is stored at 2–8 °C, it can be used for a maximum of 4 weeks. Microbial contamination must be prevented. After the expiry date, the quality guarantee is no longer valid.

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

The colorless substrate must also be protected from direct light to prevent it from decomposing or turning blue due to auto-oxidation. Once the substrate has turned blue, it must not be used.

#### 6. Additional necessary reagents – and necessary equipment

##### 6.1. Reagents

- Distilled or deionized water

##### 6.2. Equipment

- Test tubes
- Disposable pipettes (Article no.: Z0001)

- Vortex mixer (optional, see 9.3.)
- Micropipette for 50–100 µl and 1 ml volumes
- Measuring cylinder (1000 ml)
- Timer
- Washing device for microwell plates or multichannel pipettes (300 µl)
- Photometer for microwell plates (450 nm and reference filter 620–650 nm)
- Filter paper (laboratory towels)
- Waste container with 0.5% hypochlorite solution

## 7. Precaution 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. Always adhere strictly to the user instructions for this test.

Specimens or reagents must not be pipetted by mouth, and contact with injured skin or mucous membranes must be prevented. Wear personal safety gear (suitable gloves, laboratory coat, safety glasses) when handling the specimens, and wash hands after finishing the test. Do not smoke, eat, or drink in areas where samples are being processed.

For more details, refer to Material Safety Data Sheets (MSDS) at [www.r-biopharm.com](http://www.r-biopharm.com).

The kit includes a positive control that contains the inactivated giardia antigen. It must be treated as potentially infectious material and handled in accordance with the national safety regulations, just like the patient samples.

The wash buffer contains 0.1 % thimerosal as preservative. This substance must not be allowed to come into contact with skin or mucous membranes.

Ensure the proper and responsible disposal of all reagents and materials after their use. For disposal, please adhere to national regulations.

## 8. Specimen collection and storage

Until it is used, store the test material at 2–8 °C. If the material cannot be used for a test within three days, we recommend storage at -20 °C or colder. Avoid freezing and thawing the specimen repeatedly.

Stool samples and rectal smears should not be collected in transport containers which contain transport media with preservatives, animal sera, metal ions, oxidizing agents, or detergents since these may interfere with the RIDASCREEN® Giardia ELISA.

If rectal smears are used, make sure that the volume of stool material is sufficient (approx. 100 mg) for the test.

Contact tracing should include stool samples taken from contact persons who do not exhibit clinical symptoms, in order to identify asymptomatic carriers.

## 9. Test procedures

### 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 aluminum bag until they have reached room temperature. The reagents must be thoroughly mixed immediately before use. After use, the microwell strips (placed in sealed bags) and the reagents must be stored at 2-8 °C. Once used, the microwell strips must not be used again. The reagents and microwell strips must not be used if the packaging is damaged or the vials are leaking.

In order to prevent cross contamination, the samples must be prevented from coming into direct contact with the kit components.

The test must not be carried out in direct sunlight. We recommend covering the microwell plate or sealing with plastic wrap to prevent evaporation losses.

### 9.2. Preparing the wash buffer

Mix 1 part wash buffer concentrate **Wash** with 9 parts distilled water. Any crystals present in the concentrate must be dissolved beforehand by warming in a water bath at 37 °C.

### 9.3 Preparing the samples

Fill a labelled test tube with 1 ml RIDASCREEN® sample dilution buffer **Diluent | 1**. Use a disposable pipette (article no. Z0001) to aspirate a sample of thin stool (approx. 100 µl) to just above the second bulge and add this to buffer in the test tube to make a suspension. To suspend solid stool samples, use an equivalent amount of the sample (approx. 50–100 mg), handling it with a spatula or disposable inoculation loop.

Homogenize the stool suspension by aspiration into and ejection from a disposable pipette or, alternatively, blend in a Vortex mixer. Let the suspension stand a short period of time (10 minutes) for the coarse stool particles to settle; this clarified supernatant of the stool suspension can be used directly in the test. If the test procedure is carried out in an automated ELISA system, the supernatant must be particle-free. In that case, it is advisable to centrifuge the sample at 2,500 G for 5 minutes.

#### **Note:**

Stool samples diluted in **Diluent | 1** can be tested in all RIDASCREEN® ELISA for which **Diluent | 1** is used.

### 9.4. First incubation

After inserting a sufficient number of wells in the strip holder, add 100 µl of the positive control **Control | +**, the negative control **Control | -**, or the stool sample suspension to the wells. Subsequently add 100 µl of the biotin-conjugated antibody **Conjugate | 1** and blend (by tapping lightly on the side of the plate); then incubate for 30 minutes at room temperature (20–25 °C).

## 9.5. Washing

Careful washing is important in order to achieve the correct results and should therefore proceed strictly according to the instructions. The incubated substance in the wells must be emptied into a waste container and discarded in accordance with local regulations. After this, knock out the plate onto absorbent paper in order to remove the residual moisture. Then wash the plate five times using 300 µl wash buffer each time. Make sure that the wells are emptied completely by knocking them out after each wash on a part of the absorbent paper which is still dry and unused.

If you use a microplate washer or fully automated ELISA, make sure that the machine is correctly adjusted; request settings from the manufacturer, if necessary. Appliances delivered by R-Biopharm are already programmed with validated settings and work protocols. To avoid blocking the wash needles, only particle-free stool suspensions should be dispensed (see Item 9.3., Preparing the samples). Also make sure that all of the liquid is aspirated during each wash step.

## 9.6. Second incubation

Use a pipette to fill 100 µl streptavidin poly-peroxidase conjugate **Conjugate | 2** into the wells, then incubate for 15 minutes at room temperature (20–25 °C).

## 9.7. Washing

Wash as described in Item 9.5.

## 9.8. Third incubation

Fill all wells with 100 µl substrate **Substrate**. Then incubate the plate for 15 minutes in darkness at room temperature (20–25 °C). Subsequently fill all wells with 50 µl stop reagent **Stop** in order to stop the reaction. After blending cautiously by tapping lightly on the side of the plate, measure the extinction at 450 nm (optional: 450/620 nm). Adjust the zero point in the air, that is without the microwell plate.

### **Note:**

High-positive patient samples may cause black-colored precipitates of the substrate.

## **10. Quality control – indications of reagent expiry**

For quality control purposes, positive and negative controls must be used each time the test is carried out, to ensure that the reagents are stable and that the test is conducted correctly. The test has been carried out correctly if the extinction rate (OD) for the negative control is less than 0.2 at 450 nm (less than 0.160 at 450/620 nm) and the measured value for the positive control is greater than 0.8 at 450 nm or at 450/620 nm. A value greater than 0.2 (0.160) for the negative control may indicate that washing was insufficient. Deviation from the required values, just like a turbid or blue coloring of the colorless substrate before it is filled into the wells, 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 consult the manufacturer or your local R-Biopharm distributor.

## 11. Assessment and interpretation

### 11.1. Calculating the cut-off

In order to establish the cut-off, 0.15 extinction units are added to the measured extinction for the negative control.

$$\text{Cut-off} = \text{extinction for the negative control} + 0.15$$

### 11.2. Test results

Assessment of the specimen is positive if the extinction rate is more than 10 % higher than the calculated cut-off value.

Assessment of the specimen is marginal if the extinction rate ranges from 10 % less to 10 % greater than the cut-off value. If the repeat examination with a fresh stool sample again falls within the gray zone, assessment of the sample is negative.

Samples with extinctions more than 10 % below the calculated cut-off must be considered negative.

## 12. Limitations of the method

The RIDASCREEN® Giardia Test identifies the specific antigens of *Giardia lamblia* in stool samples. It is not possible to associate the determined level of extinction to the occurrence or severity of clinical symptoms. The results obtained must always be interpreted in combination with the clinical signs and symptoms.

A positive result does not rule out the presence of other infectious pathogens.

A negative result does not rule out the possibility of giardiasis. Such a result may be due to intermittent excretion of the parasite, or the amount of antigen in the sample may be too small. If the patient history supports a suspicion of *Giardia lamblia* infection, the examination should be repeated with another stool sample.

A marginal result may be due to non-homogeneous distribution of the parasites in the stool sample. In this case, examination should either be repeated with a second suspension from the same sample or another stool sample should be requested.

### 13. Performance characteristics

#### 13.1. Clinical comparison study

A clinical investigation in Great Britain evaluated the RIDASCREEN® Giardia Test with a total of 240 stool samples (prospective and blinded retrospective studies). There was a comparison with established British methods of microscopy for giardia (saline emulsion and iodine stain) as well as a differentiating real-time PCR. The results of that study are summarized in Table 1.

Table 1: Comparison of RIDASCREEN® Giardia ELISA by microscopy and by real-time PCR

		Microscopy		Real-time PCR <sup>#</sup>	
		Positive	Negative	Positive	Negative
RIDASCREEN® Giardia	Positive	11	15	26	0
	Negative	2	212	1	211

Sensitivity (CI): 85 % (55 - 98)

96 % (81 - 100)

Specificity (CI): 93 % (89 - 96)

100 % (98 - 100)

<sup>#</sup>Insufficient amount of material in two stool samples

CI: Confidence interval in %

#### 13.2 Analytical sensitivity

To determine the analytic sensitivity of the RIDASCREEN® Giardia ELISA, a linear dilution series from a sample with a known quantity of giardia cysts was produced and then measured in triplicates. The limit of detection (LoD) is the last concentration to be evaluated as positive in all replicates. The results of those measurements are shown in Table 2.

Table 2: Analytical sensitivity results for RIDASCREEN® Giardia ELISA

Cysts / Reaction	MV [OD 450/620]	Results
2.5 x 10 <sup>5</sup>	1.793	Positive
1.25 x 10 <sup>5</sup>	0.968	Positive
6.25 x 10 <sup>4</sup>	0.460	Positive
3.12 x 10 <sup>4</sup>	0.201	Positive
1.56 x 10 <sup>4</sup>	0.077	Negative
7812	0.024	Negative
3906	0.008	Negative

#### 13.3. Precision

To determine the intra-assay reproducibility, 40 replicates of these references were assayed, representing the complete measurement range from negative to high-positive. The mean values



and the variation coefficients (VC) were determined for three lots of the kits. For the inter-assay reproducibility, references from ten different working days were assayed in duplicates, with two runs per day. The measurements were determined by three technicians for three lots of the kits. The inter-lot reproducibility was determined for all three lots of the kits. The results of that study are shown in Table 3.

Table 3: Reproducibility/precision of the RIDASCREEN® Giardia ELISA

Reference		Intra-assay			Inter-assay			Inter-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1-3
1	MV [OD 450/620]	2.667	2.314	2.216	2.568	2.765	2.763	2.699
	VC (%)	5.67%	6.37%	12.03%	13.59%	11.04%	9.25%	11.85%
2	MV [OD 450/620]	2.115	1.750	1.563	1.894	2.085	2.043	2.008
	VC (%)	5.02%	8.17%	7.35%	15.08%	15.52%	12.89%	15.07%
3	MV [OD 450/620]	1.295	0.909	1.170	1.372	1.514	1.541	1.476
	VC (%)	9.52%	9.42%	20.29%	18.51%	12.92%	11.45%	15.30%
4	MV [OD 450/620]	0.829	0.683	0.817	0.880	0.956	0.983	0.940
	VC (%)	7.43%	10.59%	7.07%	16.11%	13.29%	11.95%	14.59%
5	MV [OD 450/620]	0.505	0.404	0.481	0.558	0.618	0.623	0.600
	VC (%)	8.92%	13.43%	12.97%	21.12%	18.94%	20.21%	20.49%
6	MV [OD 450/620]	0.269	0.167	0.291	0.311	0.350	0.349	0.336
	VC (%)	9.53%	16.41%	10.48%	22.22%	15.16%	21.00%	20.21%

#### 13.4. Cross reactivity

A variety of pathogenic microorganisms from the intestinal tract were examined with the RIDASCREEN® Giardia Test and showed no cross reactivity. These studies were conducted with undiluted bacteria or virus suspensions shown to have concentrations of  $10^6$  to  $10^9$  organisms per ml. The results of that study are listed in Table 4.

Table 4: Cross reactivity with pathogenic microorganisms

Organism	Origin	MV [OD 450/620]
<i>Adenovirus</i>	Cell culture supernatant	-0.005
<i>Aeromonas hydrophila</i>	Culture	-0.001
<i>Arcobacter butzleri</i>	Culture	-0.003
<i>Astrovirus</i>	Cell culture supernatant	-0.001

<i>Bacillus cereus</i>	Culture	0.001
<i>Bacteroides fragilis</i>	Culture	-0.002
<i>Campylobacter coli</i>	Culture	0.001
<i>Campylobacter fetus subsp.</i>	Culture	0.001
<i>Campylobacter jejuni</i>	Culture	0.001
<i>Campylobacter lari subsp.</i>	Culture	0.006
<i>Campylobacter upsaliensis</i>	Culture	0.001
<i>Candida albicans</i>	Culture	-0.001
<i>Citrobacter freundii</i>	Culture	0.000
<i>Clostridium bifermentans</i>	Culture	-0.002
<i>Clostridium difficile</i>	Culture	-0.003
<i>Clostridium novyi</i>	Culture	-0.004
<i>Clostridium perfringens</i>	Culture	-0.001
<i>Clostridium septicum</i>	Culture	-0.005
<i>Clostridium sordellii</i>	Culture	-0.001
<i>Clostridium sporogenes</i>	Culture	-0.006
<i>Cryptosporidium muris</i>	Culture	-0.005
<i>Cryptosporidium parvum</i>	Culture	-0.003
<i>E. coli</i> (EPEC)	Culture	0.001
<i>E. coli</i> (ETEC)	Culture	0.002
<i>E. coli</i> (STEC)	Culture	0.000
<i>Entamoeba histolytica</i>	Culture	-0.002
<i>Enterobacter cloacae</i>	Culture	0.002
<i>Enterococcus faecalis</i>	Culture	-0.001
<i>Klebsiella oxytoca</i>	Culture	0.001
<i>Proteus vulgaris</i>	Culture	0.000
<i>Pseudomonas aeruginosa</i>	Culture	0.006
<i>Rotavirus</i>	Cell culture supernatant	-0.005
<i>Salmonella enteritidis</i>	Culture	0.000
<i>Salmonella typhimurium</i>	Culture	0.000
<i>Serratia liquefaciens</i>	Culture	-0.001
<i>Shigella flexneri</i>	Culture	0.003
<i>Staphylococcus aureus</i>	Culture	0.001
<i>Staphylococcus epidermidis</i>	Culture	0.001
<i>Vibrio parahaemolyticus</i>	Culture	-0.001
<i>Yersinia enterocolitica</i>	Culture	0.000

### 13.5 Interfering substances

The following list of substances showed no effects on the test results when they were blended into giardia positive and giardia negative stool samples in the described concentrations:

barium sulfate (18.5% w/w), loperamide (antidiarrheal drug; 0.02% w/w), Pepto-Bismol (antidiarrheal drug, 6.6% v/w), mucins (5.0% w/w), cyclamate (artificial sweetener, 1.3% v/w), human blood (5.0% v/w), stearic acid and palmitinic acid (mixture 1:1, 40.0% w/w), metronidazole (0.5) (antibiotic 3.0% v/w), diclofenac (0.1% v/w).

## Appendix

Test specific symbols:

Plate	Microwell plate
Diluent   1	Sample dilution buffer
Wash	Wash buffer
Control   +	Positive control
Control   -	Negative control
Conjugate   1	Conjugate 1
Conjugate   2	Conjugate 2
Substrate	Substrate
Stop	Stop reagent

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