

# **RIDASCREEN<sup>®</sup> Entamoeba histolytica IgG**

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## 1. Intended use

For *in vitro* diagnostic use. The RIDASCREEN® Entamoeba histolytica IgG test is an enzyme immunoassay for the qualitative determination of IgG antibodies against Entamoeba histolytica in human serum.

The test should be used for confirmation purposes when there is a suspected case of amoebiasis.

## 2. Summary and explanation of the test

Entamoeba histolytica is a human pathogenic protozoon and one of the most common parasites in developing countries, especially in the tropical and subtropical areas.<sup>4</sup> Annually about 50 million people are infected of which 100,000 die as a result of the infection.<sup>1</sup> Typically, the pathogen is transmitted through the faecal-oral route. In this way the infectious cysts are absorbed. By reaching the small intestine, they develop into the vegetative trophozoites that subsequently colonize the colon.<sup>3</sup> Through invasion of the parasite from the gut lumen into the colon mucosa, 10% of infected patients develop an invasive amoebiasis.<sup>5</sup> The incubation period is highly variable, therefore the incubation can take a few days but also months or even years. In case of an intestinal amoebiasis the incubation period generally takes 1-4 weeks.<sup>11</sup>

Clinical symptoms of an intestinal amoebiasis are crampy abdominal pain accompanied by nausea and severe diarrhea with bloody and mucous stool. During acute dysentery, extraintestinal complications such as liver abscesses may occur caused by haematogenic spread which can lead to death if left untreated.<sup>3</sup>

After infection with Entamoeba histolytica, 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 those antibodies in the serum. Apart from the appropriate choice of the pathogen-specific antigen, also the selected test method has a great impact on the result of the 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 step by using enzyme-labelled Protein A (the conjugate). The enzyme converts the colourless substrate (urea peroxide/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

There are enough reagents in the kit for 96 determinations.

<b>Plate</b>	96 det.	Microwell plate, 12 microwell strips (can be divided) in strip holder; coated with antigens from <i>Entamoeba histolytica</i>
<b>Diluent</b>	100 ml	Sample buffer, phosphate-buffered NaCl solution, ready for use; coloured yellow
<b>SeroWP</b>	100 ml	Wash buffer, 10-fold concentrate; tris-buffered NaCl solution;
<b>Control +</b> <i>red lid</i>	1.2 ml	IgG positive control, human serum, ready for use;
<b>Control -</b> <i>colourless lid</i>	2.5 ml	IgG negative control, human serum, ready for use
<b>Conjugate</b> <i>orange lid</i>	12 ml	Protein A-conjugate, ready for use; peroxidase conjugated Protein A in stabilized protein solution
<b>SeroSC</b>	12 ml	Substrate H <sub>2</sub> O <sub>2</sub> /tetramethylbenzidine; ready for use
<b>Stop</b>	12 ml	Stop reagent 0.5 M sulphuric acid; ready for use

Details of hazardous substances according to labeling obligations. For more details see Material Safety Data Sheets (MSDS) at [www.r-biopharm.com](http://www.r-biopharm.com).

## 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 stored in the aluminium bag.

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

## 6. Materials required but not provided

### 6.1. Reagents

- distilled or deionised water

### 6.2. Accessories

- 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  $\geq$  620 nm)
- Filter paper (laboratory towels)
- Waste container containing 0.5 % 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 have to be followed. The instruction manual for the test procedure has to be followed. Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes. During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure. Do not smoke, eat or drink in areas where samples or reagents are being used.

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

The control sera (positive 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.

All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.

## 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 1: 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 between 2 and 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.

The wash buffer, sample buffer and substrate are not test specific; they can also be used for other RIDASCREEN® ELISA for determining antibodies against parasites.

### 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 buffer can be used for a maximum of 4 weeks provided it is 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 **Diluent** 1:50 before starting the test.

e.g. 10 µl Serum + 490 µl **Diluent**

#### Note:

**The negative control and positive control are ready for use and must NOT be diluted.**

#### 9.4. First incubation

After inserting a sufficient number of wells in the frame, pipette 100 µl diluted sera and ready-to-use controls **Control -** and **Control +** into each of the corresponding wells and incubate at room temperature (20 – 25 °C) for 15 minutes. We recommend that you carry out the negative control **Control -** in duplicate.

#### 9.5. Washing

The wells must be emptied into a waste container containing hypochlorite solution for disinfection. After this, knock out the plate onto absorbent paper in order to remove the residual moisture. After this, wash the plate 5 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 of the protein A-conjugate **Conjugate** to each well. Then incubate the plate at room temperature (20 – 25 °C) for 15 minutes.

#### 9.7. Washing

Wash 5 times in accordance with Section 9.5.

#### 9.8. Third incubation

Place 100 µl substrate **SeroSC** in each the well. Then incubate the plate at room temperature (20 – 25 °C) for 15 minutes. After this, stop the reaction by adding 50 µl stop reagent **Stop** 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 zero against air.

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

For quality control purposes, the positive control and the negative control (in duplicate) must be used every time the test is carried out. The test has been carried out correctly if the average extinction of the negative control at 450 nm is smaller than 0.3. If the two individual measurements deviate from the average by more than 25 %, the test must be repeated. The extinction for the positive control at 450 nm must be greater than 0.8.

If the values differ from those required, if the substrate is turbid or 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

### 11.1. Calculating the sample index

1. The average absorbance is calculated for the negative control.
2. 0.150 is added to the average absorbance. This yields the cut-off for the test.
3. The sample index is obtained by dividing the absorbance for the sample by the cut-off.

For example:    Negative control 1        O.D. = 0.115  
                      Negative control 2        O.D. = 0.125  
                      Sample                            O.D. = 0.508

$$\text{cut-off} = \frac{0.115 + 0.125}{2} + 0.150 = 0.270$$

$$\text{Sample index} = \frac{0.508}{0.270} = 1.88$$

### 11.2. Test result

Table 2: Evaluating the sample index

	negative	equivocal	positive
Sample index	< 0.9	0.9 – 1.1	> 1.1

## 12. Limitations of the method

RIDASCREEN® Entamoeba histolytica IgG ELISA detects IgG antibodies against Entamoeba histolytica. and should be carried out in cases of suspected Amoebiasis. The results obtained must always be interpreted in combination with the clinical picture and other diagnostic findings.

Antibody signals are dependent on the localization of the parasitosis and may vary from patient to patient.

If the amoebae findings in the stools are positive, a positive antibody determination will mean that Entamoeba histolytica can be identified in the strict sense as the cause of the clinical symptoms.

In cases of suspected extraintestinal dispersal of the parasite, a positive antibody determination can indicate amoebiasis, even if the stool findings are negative; the findings should be confirmed by clinical symptoms and other diagnostic methods.

A negative result does not necessarily rule out the possibility of amoebiasis. During the early stages of the infection, the number of antibodies may be so small that the test yields a negative or equivocal result. In cases of suspected amoebiasis based on the case history, another serum sample should be tested after a few days.

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

## 13. Performance characteristics

Table 3: Inter-assay variation (n = 30)

<u>Inter-assay variation</u>	<u>IgG</u>	
	index	CV
Serum 1	4.52	7.1 %
Serum 2	2.63	5.1 %
Serum 3	2.42	10.1 %
Serum 4	0.24	n/a



Table 4: Intra-assay variation (n = 23)

<u>Intra-assay variation</u>	<u>IgG</u>	
	index	CV
Serum 1	4.40	12.3 %
Serum 2	1.78	4.7 %
Serum 3	1.54	3.5 %
Serum 4	0.32	n/a

Table 5: Sensitivity and specificity in comparison with one other commercial ELISAs

	<u>IgG</u>
Sensitivity	100.0%
Specificity	100.0%

Table 6: Results from testing 200 blood-donor sera taken from a blood donor centre in Germany

	negative	equivocal	positive
200 blood donor sera	96.5 %	2.0 %	1.5 %

## References

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