IBL INTERNATIONAL

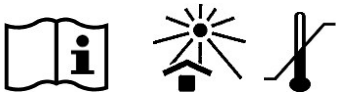
Instructions for Use

Entamoeba histolytica IgG ELISA

Enzyme immunoassay for the in-vitro-diagnostic qualitative determination of IgG antibodies against Entamoeba histolytica in human serum or plasma.

REF **RE58631**

Σ **96**



EU: IVD CE U.S.: *For research use only.*
Not for use in diagnostic procedures.

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1. INTRODUCTION

Entamoeba histolytica is an anaerobe parasite forming cysts which have four small nuclei and measure 10-15 µm in diameter. The cysts are sturdy and resist adverse environmental conditions. After ingestion by a susceptible host (invertebrates and vertebrates including humans), its wall is disrupted by the formation of a small opening through which an amoeba emerges. The amoeba divides serially through three cycles giving rise to eight uninucleate trophozoites from one cyst which are motile and measure 20-30 µm in diameter. Some of the trophozoites then invade the tissues of the large intestine and may erode them so extensively that they gain entrance into the bloodstream. Thus, amoebae can reach all parts of the body. Infection with Entamoeba histolytica has worldwide distribution. It is the causative agent of amoebiasis and amoebic dysentery and inhabits the lumen and mucosa of the large intestine, predominantly the transverse colon and cecum. Extra intestinal amoebiasis can afflict any organ or tissue. The majority of infected individuals are free of symptoms; this high incidence of asymptomatic carriers complicates matters. Those who are symptomatic experience a wide range of manifestations. Members of all age groups and both sexes are infected. The risk of infection increases with inadequate sanitary conditions. An increased prevalence of amoebiasis is found among people, who have an increased risk of exposure in the agricultural occupations and in male homosexuals.

Species	Disease	Symptoms	Mechanism of Infection
Entamoeba histolytica	Amoebiasis Dysentery Amoebic liver abscess, brain abscess Hepatic amoebiasis Carcinoma of the colon	Non specific diarrhoea ulcerative colitis abdominal cramps, bloody stools, dysentery	Transmission through faeces or contaminated food/water by oral ingestion of cysts. Sexual transmission among male homosexuals

Infection may be identified by

- Microscopy: stool examination: iron haematoxylin method, merthiolat iod formol concentration (MIFC)
- Serology: CF, CIE, ELISA

2. INTENDED USE

The Entamoeba histolytica IgG-ELISA is intended for the qualitative determination of antibodies against Entamoeba histolytica antigens in human serum or plasma (citrate). Especially asymptomatic carriers and cases of a previous non-invasive amoebiasis may be identified.

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of antibodies against Entamoeba histolytica is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Especially asymptomatic carriers and cases of a previous non-invasive amoebiasis may be identified.
Microtiter strip wells are precoated with Entamoeba histolytica antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled Protein A Conjugate is added. This conjugate binds to the captured Entamoeba histolytica 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 Entamoeba histolytica specific 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

- **Entamoeba histolytica Coated Wells:** 12 breakapart 8-well snap-off strips coated with Entamoeba histolytica 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 (pH 7.2 ± 0.2) for washing the wells; white cap.
- **Entamoeba histolytica Protein A conjugate**:** 1 bottle containing 20 mL of peroxidase Protein A; coloured blue, ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.
- **Entamoeba histolytica Positive Control***:** 1 bottle containing 2 mL; coloured yellow; ready to use; red cap.
- **Entamoeba histolytica Cut-off Control***:** 1 bottle containing 3 mL; coloured yellow; ready to use; green cap.
- **Entamoeba histolytica Negative Control***:** 1 bottle containing 2 mL; coloured yellow; ready to use; blue 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

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- 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 controls 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 Entamoeba histolytica 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. Entamoeba histolytica Protein A Conjugate

The bottle contains 20 mL of a solution with Protein A, horseradish peroxidase, buffer, stabilizers, preservatives and an inert blue dye. Protein A is an immunoglobulin Fc-binding protein with a molecular weight of 42,000 Daltons. The solution is ready to use. Store at 2...8°C. *After first opening stability until expiry date when stored at 2…8 °C.*

6.3. Controls

The bottles labelled with Positive, Cut-off and Negative Control contain a ready to use control solution. It has to be stored at 2...8°C. *After first opening stability 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 stability until expiry date when stored at 2…8 °C.*

6.5. Washing Solution (20x conc.)

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 have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be discharged*. *After first opening stability 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. *Do not heat inactivate the serum and 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 1 mL 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 350µLt 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,
	1 well	(e.g. B1)	for the negative control,
	2 wells	(e.g. C1+D1)	for the cut-off control and
	1 well	(e.g. E1)	for the positive control.

It is recommended to determine controls and patient samples in duplicate, if necessary.

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 controls and diluted samples into their 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 of 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 >5 sec. 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 Entamoeba histolytica Protein A Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature.** *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 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 sample 1+100 with IgG Sample Diluent and multiply the results in Units = U by 2.

11. Measure the absorbance of the specimen at 450/620 nm 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. Run Validation Criteria

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

- **Substrate blank** in A1: Absorbance value **< 0.100.**
- **Negative control** in B1: Absorbance value **< 0.200 and < cut-off**
- **Cut-off control** in C1 and D1: Absorbance value **0.150 – 1.30.**
- **Positive control** in E1: Absorbance value **> cut-off**.

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

9.2. Calculation of Results

The cut-off is the mean absorbance value of the Cut-off control determinations.

Example: Absorbance value Cut-off control 0.42 + absorbance value Cut-off control 0.44 =0.86 / 2 = 0.43

Cut-off = 0.43

9.3. Interpretation of Results

Samples are considered **POSITIVE** if the absorbance value is higher than 10% over the cut-off.

Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative

→ **grey zone**

It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample has to be considered **NEGATIVE**.

Samples are considered **NEGATIVE** if the absorbance value is lower than 10% below the cut-off.

9.3.1. Results in Units

$$\frac{\text{Patient (mean) absorbance value} \times 10}{\text{Cut-off}} = [\text{Units} = \text{U}]$$

Example: $\frac{1.376 \times 10}{0.43} = 32$ *TU (Units)*

Cut-off:	10	U
Grey zone:	9-11	U
Negative:	<9	U
Positive:	>11	U

10. SPECIFIC PERFORMANCE CHARACTERISTICS

10.1. Precision

Interassay	**n**	**Mean**	**Cv (%)**
Pos. Serum	6	0.604	3.9
Intraassay	**n**	**Mean**	**Cv (%)**
Pos. Serum	7	0.609	2.1

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 >95 %.

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 >95 %.

10.4. Interferences

Interferences with lipemic or icteric sera are not observed up to a concentration of 5 mg/mL triglycerides and 0.2 mg/mL bilirubin. Hemoglobin could show cross reactivity in negative sera.

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 immunocompromised 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 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.

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SCHEME OF THE ASSAY

Entamoeba histolytica IgG ELISA

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate blank (e.g. A1)	Negative control	Positive control	Cut-off control	Sample (diluted 1+100)
Negative control	-	100µL	-	-	-
Positive control	-	-	100µL	-	-
Cut-off control	-	-	-	100µL	-
Sample (diluted 1+100)	-	-	-	-	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
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	100µL	100µL	100µL	100µL	100µL
Incubate for exactly 15 min at room temperature in the dark					
Stop Solution	100µL	100µL	100µL	100µL	100µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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