



AccuDiag™ E. histolytica IgG ELISA Kit

REF 8201

PIC PB8201TX44

IVD See External Label 2°C 8°C 96 Tests

E. histolytica IgG ELISA

Principle	Indirect ELISA
Detection Range	Qualitative
Sample	10 µL serum/plasma
Incubation Time	105 minutes
Shelf Life	12 Months from the manufacturing date
Specificity	98%
Sensitivity	100%

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Entamoeba histolytica IgG ELISA is intended for the qualitative determination of IgG class antibodies against Entamoeba histolytica in human serum or plasma (citrate, heparin).

ASSAY PRINCIPLE

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter plates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This

conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. 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 specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance at 450/620 nm is read using an ELISA Microtiter plate reader.

SPECIMEN COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2-8°C; otherwise, they should be aliquoted and stored deep-frozen (-70-20°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivation of samples is not recommended.

1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with Sample Dilution Buffer. Dispense 10 µL sample and 1 mL Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

REAGENTS

Reagents provided with the test kit

- Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Entamoeba histolytica antigens; in resealable aluminium foil.
- Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; colored yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- Wash Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap; 0.2% (w/v) 5-Bromo-5-nitro-1,3-dioxane.
- Conjugate:** 1 bottle containing 20 mL of peroxidase labeled antibody to human IgG in phosphate buffer (10 mM); colored blue; ready to use; black cap.
- TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1%; ready to use; yellow cap.
- Positive Control:** 1 vial containing 2 mL control; colored yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- Cut-off Control:** 1 vial containing 3 mL control; colored yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- Negative Control:** 1 vial containing 2 mL control; colored yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see "Precautions and Warnings"

Materials provided with the test kit

- 1 Cover foil
- 1 Instructions for use (IFU)

Materials required but not provided

- ELISA Microtiter plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing Microtiter plates
- Pipettes to deliver volumes between 10 and 1000 µL



5. Vortex tube mixer
6. Distilled water
7. Disposable tubes

REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20-25°C) and mix them before starting the test run

1. **Microtiter plate**
The break-apart snap-off strips are coated with Entamoeba histolytica antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminum foil along with the desiccant supplied and stored at 2-8°C.
2. **Wash Buffer (20x conc.)**
Dilute Washing Buffer 1 + 19; e.g. 10 mL Wash Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20-25°C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.
3. **TMB Substrate Solution**
The reagent is ready to use and has to be stored at 2-8°C, away from the light. The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

ASSAY PROCEDURE

Please read the instructions for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instructions for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of the Wash Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter "Precautions and Warnings". Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays. A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1°C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 60 min ± 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 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper before the next step!
Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20-25°C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20-25°C) in the dark.** A blue color occurs due to an enzymatic reaction.

10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a color change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after the addition of the Stop Solution.

Measurement

Adjust the ELISA Microtiter plate reader **to zero** using the **Substrate Blank**. If - due to technical reasons - the ELISA Microtiter plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value 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 standard/control and sample.

Biochromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

RESULTS

1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

Substrate Blank: Absorbance value < **0.100**

Negative Control: Absorbance value < **0.200** and < **Cut-off**

Cut-off Control: Absorbance value **0.150 – 1.300**

Positive Control: Absorbance value > **Cut-off**

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

2. Calculation of Results

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

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

Cut-off = 0.43

Results in Units [U]

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

Example:
$$\frac{1.591 \times 10}{0.43} = 37 \text{ U}$$

3. Interpretation of Results

Cut-off	10 U	-
Positive	> 11 U	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 U	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks.
Negative	< 9 U	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.

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.



PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

1. Precision

Intra-assay	n	Mean (E)	CV (%)
#1	24	0.611	4.50
#2	24	0.999	9.29
#3	24	1.186	7.78

Inter-assay	n	Mean (U)	CV (%)
#1	12	17.99	7.73
#2	12	27.58	11.51
#3	12	3.125	14.26

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 98.0% (95% confidence interval: 92.96% - 99.76%).

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 100% (95% confidence interval: 91.19% - 100%).

4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false positive results due to cross-reactions.

- 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 standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to "Reagents"). Therefore, the following hazard and precautionary statements apply.

Warning



H317
P261
P280
P302+P352
P333+P313
P362+P364

May cause an allergic skin reaction.
Avoid breathing spray.
Wear protective gloves/protective clothing.
IF ON SKIN: Wash with plenty of soap and water.
If skin irritation or rash occurs: Get medical advice/attention.
Take off contaminated and Wash it before reuse.

Reagents may contain 5-Bromo-5-nitro-1,3-dioxane (refer to "Reagents") Therefore, the following hazard and precautionary statements apply.

Warning



H315
H319
P280
P302+P352
P305+P351+P338
P337+P313

Causes skin irritation.
Causes serious eye irritation.
Wear protective gloves/protective clothing.
IF ON SKIN: Wash with plenty of soap and water.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
If eye irritation persists: Get medical advice/attention.

Further information can be found in the safety data sheet.

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.

STABILITY AND STORAGE

Store the kit at 2-8°C. The opened reagents are stable up to the expiry date stated on the label when stored at 2-8°C.

LIMITATIONS OF THE ASSAY

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.


PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits 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 materials of human or animal origin should be regarded and handled as potentially infectious.
- 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.
- Do not interchange reagents or Microtiter plates of different production lots.



MANUFACTURER AND BRAND DETAILS

ISO 13485:2016



Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

Date Adopted	2024-06
Brand Name	AccuDiag™
REF 8201	AccuDiag™ - <i>E. histolytica</i> IgG ELISA
PIC	PB8201TX44

Revision Date: 2020-07-23

SUMMARY OF THE ASSAY

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all samples and standards/controls on the plate layout supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Negative Control	Cut-off Control	Positive Control	Sample (diluted 1:100)
Negative Control	-	100 µL	-	-	-
Cut-off Control	-	-	100 µL	-	-
Positive Control	-	-	-	100 µL	-
Sample (diluted 1+100)	-	-	-	-	100 µL
Cover wells with foil supplied in the kit Incubate for 60 min at 37 ± 1°C Wash each well three times with 300 µL of Washing Buffer					
Conjugate	-	100 µL	100 µL	100 µL	100 µL
Incubate for 30 min at room temperature (20-25°C) Do not expose to direct sunlight Wash each well three times with 300 µL of Washing Buffer					
TMB Substrate Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Incubate for exactly 15 min at room temperature (20-25°C) in the dark					
Stop Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)					