

ab178643 – Giardia lamblia ELISA Kit

Instructions for Use

For the qualitative measurement of Giardia lamblia antigens in faeces.

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Abcam's Giardia lamblia in vitro ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate qualitative measurement of Giardia lamblia antigens in faeces.

A 96-well plate has been precoated with polyclonal anti-Giardia lamblia antibodies. Controls or test samples are added to the wells and incubated; Giardia antigens present in the stool supernatant are captured. Following washing, a horseradish peroxidase (HRP) labelled anti-Giardia antibody is added to the wells, which binds to the immobilized antibodies. TMB is then catalyzed by the HRP to produce a blue color product that changes to yellow after adding an acidic stop solution. The density of yellow coloration is directly proportional to the amount of Giardia lamblia antigen sample captured in plate.

Giardia lamblia is one of the most common human intestinal protozoan pathogens worldwide. The incidence strongly depends on the geographic region and reaches 2-7 % in central Europe and exceeds 50 % in tropical countries. The life cycle of Giardia lamblia is characterized by two stages: the trophozoite and the cyst stage. The trophozoite is the motile dividing stage and inhabits the upper small intestine. Ascending infections of the gallbladder may also occur. The cyst is the infective form of the parasite. It develops in the intestine and is excreted with the faeces. Cysts are transmitted via contaminated food or drinking water but also from person to person. The clinical picture of a Giardia lamblia infection ranges from the asymptomatic carrier state to acute diarrhea which is often accompanied by abdominal pain and flatulence. Chronic giardiasis can cause severe malabsorption syndrome. Giardiasis is usually diagnosed by microscopic detection of trophozoites and/or cysts in faecal smears after commonly used staining techniques or direct immune fluorescence. These methods are time-consuming, require trained personnel and can only detect parasites with intact morphology. Immunologic methods like enzyme immunoassays detecting Giardia lamblia antigens may overcome these problems.

2. ASSAY SUMMARY

Primary capture antibody



Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.

Sample



Add standard or sample to each well used. Incubate at room temperature.

HRP conjugated antibody



Aspirate and wash each well. Add prepared HRP labeled secondary detector antibody. Incubate at room temperature

Substrate **Colored product**



Aspirate and wash each well. Add TMB Substrate Solution to each well. Add stop solution and read absorbance at 450 nm.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition
Anti-Giardia lamblia Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
Sample Diluent	100 mL	2-8°C
Stop Solution	15 mL	2-8°C
10X Washing Solution	100 mL	2-8°C
Anti-Giardia lamblia HRP Conjugate	12 mL	2-8°C
TMB Substrate Solution	15 mL	2-8°C
Giardia lamblia Antigen Positive Control	2 mL	2-8°C
Giardia lamblia Antigen Negative Control	2 mL	2-8°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Incubator at 37°C
- Multi and single channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- 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
- 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

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 1X Washing Solution

Prepare 1X Washing Solution by diluting 10X Washing Solution with deionized water. To make 200 mL 1X Washing Solution combine 20 mL 10X Washing Solution with 180 mL deionized water. Mix thoroughly and gently.

- All other solutions are supplied ready to use.

10. SAMPLE COLLECTION AND STORAGE

The test is intended for the detection of *Gardia lamblia* in diluted stool specimen. Either fresh or frozen specimen may be used in this test. If samples are stored frozen, thaw sample quickly, warm to room temperature and mix thawed samples well before dilution. If the assay is performed within 72 hours after sample collection, the specimen should be kept at 2-8°C; otherwise they should be aliquoted and stored deep-frozen (-20°C). *Avoid repeated freezing and thawing.*

11. SAMPLE PREPARATION

Before assaying, all samples should be diluted 1:100 with Sample Diluent. Add 1000 µL sample into a clean tube. Using a disposable stirring rod transfer about 100 mg (about 2-3 mm diameter) of faeces if solid or pipette 100 µL if liquid into the tube with sample diluent and suspend thoroughly, e.g. on a vortex. Allow floating particles to sediment for 10 min at most. If a stool suspension sediments longer than 10 min the sample should be mixed again immediately before starting the assay.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition.
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates).

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Reliability of results depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 μ L to 350 μ L to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**
 - 13.1. Prepare all reagents, standards, and samples as directed in the previous sections.
 - 13.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
 - 13.3. Add 75 μ L of controls and 100 μ L diluted stool sample (supernatant) into appropriate wells. Leave one well for substrate blank.
 - 13.4. Cover wells with the foil supplied in the kit and incubate for 30 minutes at room temperature.
 - 13.5. Remove the foil, aspirate the contents of the wells and wash each well five times with 400 μ L of 1X Washing Solution. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining 1X Washing Solution by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.

Note: Complete removal of liquid at each step is essential for good assay performance.
 - 13.6. Add 75 μ L Anti-Giardia lamblia HRP Conjugate into all wells except for the blank well. Cover with foil.

- 13.7. Incubate for 30 minutes at room temperature. Do not expose to direct sunlight.
- 13.8. Repeat step 13.5.
- 13.9. Add 75 μ L TMB Substrate Solution into all wells
- 13.10. Incubate for exactly 10 minutes at room temperature in the dark.
- 13.11. Add 75 μ L Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
Note: Any blue color developed during the incubation turns into yellow.
- 13.12. Measure the absorbance of the specimen at 450 nm within 30 minutes of addition of the Stop Solution.
Dual wavelength reading using 620 nm as reference wavelength is recommended.

14. CALCULATIONS

Validation Criteria

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

- **Negative control:** Absorbance value < **0.200**
- **Positive control:** Absorbance value > **0.800**

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

Calculation of Results

Calculate the mean background subtracted absorbances for each sample and compare to mean Cut-off control value.

The cut-off is calculated by addition of 0.20 absorbance units to the measured absorption of the mean value of the two negative control determinations.

Example: $0.12 \text{ OD neg. control} + 0.14 \text{ OD neg. control} = 0.26 \div 2 = 0.13$

Cut-off = absorbance mean value of the negative control + 0.20

Mean Cut Off value = $0.13 + 0.20 = 0.33$

Interpretation of Results

Samples are considered to give a positive signal if the absorbance value is greater than 10% over the cut-off value.

Samples with an absorbance value of less than 10% above or below the Cut-off control value should be considered as inconclusive (grey zone) i.e. neither positive or negative. It is recommended to repeat the assay using fresh samples. If results of the second test are again less than 10% above or below the Cut-off control value the sample has to be considered negative.

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

15. TYPICAL SAMPLE VALUES

PRECISION –

Intra-Assay	n	Mean	%CV
Sample 1	12	1.438	6.86
Sample 2	12	1.007	6.41
Sample 3	12	0.637	6.36
Sample 4	12	0.246	5.04

Inter-Assay	n	Mean	%CV
Sample 1	11	1.488	4.77
Sample 2	11	1.034	4.64
Sample 3	11	0.656	7.77
Sample 4	11	0.246	10.9

16. ASSAY ANALYTICAL SPECS

SPECIFICITY –

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. 369 known negative samples were tested with this assay in comparison to IFT resulting in a specificity of 99.5% (367/369).

SENSITIVITY –

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. 40 known positive samples were tested with this assay in comparison to IFT resulting in a sensitivity of 97.5% (39/40).

LOWER DETECTION LIMIT –

The lower detection limit of Giardia lamblia antigen was determined by titration of faecal samples spiked with Giardia lamblia cysts and trophozoites from culture. The lower detection limit for Giardia lamblia

was determined 5×10^3 cysts and 2.5×10^4 trophozoites per ml of diluted faecal sample.

CROSS REACTIVITY –

Faecal samples positive for one of the following intestinal parasites and other pathogens resp., did not show any cross reactions in this assay:

Adenovirus
Ancylostoma duodenale
Ascaris lumbricoides
Blastocystis hominis
Cryptosporidium parvum
Entamoeba dispar
Entamoeba histolytica
Rotavirus

Negative stool samples have been spiked with >10⁸ colony forming units of the following microorganisms and tested negative with the Giardia lamblia ELISA kit.

Aeromonas hydrophila	Clostridium sordellii	Pseudomonas aeruginose
Bacillus cereua	Enterobacter aerogenes	Salmonella enterica Serovar enteritidis
Bacillus subtilis	Enterobacter cloacae	Salmonella enterica Serovar typhimurium
Bacteroides fragilis	Enterococcus faecalis	Shigella flexneri
Candida albicans	Escherichia coli	Shigella sonnei
Campylobacter coli	Klebsiella pneumoniae	Staphylococcus aureus
Campylobacter jejuni	Peptostreptococcus anaerobius	Staphylococcus epidermidis
Citrobacter freundii	Proteus vulgaris	Vibrio parahaemolyticus

17. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time too short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

RESOURCES

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

18. NOTES

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