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Fecal Giardia *lamblia* Antigen ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Detection of Giardia *lamblia* Antigen in Feces

REF **KTR- 838** Σ **96** +2 +8 °C

For Research Use Only **Not for Use in Diagnostic Procedures**

INTENDED USE

This microplate-based ELISA (enzyme linked immunosorbent assay) kit is intended for the qualitative detection of *Giardia lamblia* antigen in feces. The assay is a useful tool in the diagnosis of active *Giardia lamblia* infection in acute or chronic gastroenteritis.

SUMMARY OF PHYSIOLOGY

Giardia lamblia (also known as *Giardia intestinalis*) has a characteristic tear-drop shape and measures 10-15 µm in length. It has twin nuclei and an adhesive disk which is a rigid structure reinforced by supelicular microtubules. There are two median bodies of unknown function, but their shape is important for differentiating between species. There are 4 pairs of flagella, one anterior pair, two posterior pairs and a caudal pair. These organisms have no mitochondria, endoplasmic reticulum, golgi, or lysosomes. *Giardia* has a two-stage life cycle consisting of trophozoite and cyst. The life cycle begins with ingested cysts, which release trophozoites (10-20 µm x 5-15 µm) in the duodenum. These trophozoites attach to the surface of the intestinal epithelium using a ventral sucking disk and then reproduce by binary fission. The trigger for encystment is unclear, but the process results in the inactive, environmentally resistant form of Giardia -- a cyst (11-14 µm x 7-10 µm) that is excreted in feces.

Giardiasis is a diarrheal illness caused by *Giardia lamblia*, after ingestion of *Giardia* cysts. Once a person has been infected with *Giardia*, the parasite lives in the intestine and is passed in the stool. Millions of germs can be released in a bowel movement from an infected human or animal. *Giardia* is found in soil, food, water, or surfaces that have been contaminated with the feces from infected humans or animals. Because the parasite is protected by an outer shell, it can survive outside the body and in the environment for long periods of time.

Because it is spread world-wide, Giardia lamblia has become one of the most important causes of chronic diarrhea. About 15-20% of children under age ten years and 19% of male homosexuals have been infected. *Giardia* infection can cause a variety of intestinal symptoms either acute or chronic, which include diarrhea, gas or flatulence, greasy stools that tend to float, stomach cramps, upset stomach or nausea. These symptoms may lead to weight loss and dehydration. Some people with giardiasis have no symptoms at all. Those asymptomatic cases still shed *Giardia* cysts. Generally, symptoms of giardiasis begin 1 to 2 weeks after becoming infected and they may last 2 to 6 weeks.

The method used for the diagnosis of giardiasis in the past has been the detection of *Giardia* cysts in stool by microscopy. Recently, specific *Giardia* antigen ELISA greatly simplified the diagnostic procedure and is as sensitive as the microscopic method. Another advantage of using *Giardia* antigen ELISA is that it does not require the intact organisms in the test specimen.

ASSAY PRINCIPLE

This "sandwich" ELISA is designed, developed and produced for the qualitative measurement of *Giardia lamblia* antigen in stool specimen. The assay utilizes the microplate-based enzyme immunoassay technique by coating highly purified antibody onto the wall of microtiter well. Assay controls and fecal specimen are added to microtiter wells of microplate that was coated with a highly purified polyclonal anti-*Giardia lamblia* antibody on its wall. The *Giardia lamblia* antigen will be bound to the antibody coated plate after an incubation period. The unbound matrices are washed away and a HRP-conjugated monoclonal antibody which specifically recognizes the protein of *Giardia lamblia* is added for further immunoreactions. After an incubation period, an immunocomplex of "Anti-*Giardia* Antibody – *Giardia lamblia* Antigen – HRP-conjugated Anti-*Giardia* Tracer Antibody" is formed if *Giardia lamblia* antigen is present in the test sample. The unbound tracer antibody and other protein or buffer matrix are removed in the subsequent washing step. HRP-conjugated tracer antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to giardia proteins captured on the wall of each microtiter well is directly proportional to the amount of *Giardia lamblia* antigen level in each test specimen.

REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.

1. **Anti-*Giardia* Antibody Coated Microplate (Cat. No. 30190)**
   One microplate with 12 x eight strips (96 wells total) coated with highly purified anti-*Giardia* antibody. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. **Anti-*Giardia* Tracer Antibody (Cat. No. 30191)**
   One vial containing 0.6 mL concentrated horseradish peroxidase (HRP)-conjugated monoclonal *Giardia* antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. **Tracer Antibody Diluent (Cat. No. 30017)**
   One vial containing 12 mL ready-to-use buffer. It should be only used for antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. ELISA HRP Substrate (Cat. No. 10020)
One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

5. ELISA Stop Solution (Cat. No. 10030)
One bottle contains 12 mL of 0.5 M sulfuric acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

6. *Giardia* Antigen Controls (Cat. No. 30192 – 30193)
One vial contains *Giardia* negative control (30192) and another vial contains inactivated *Giardia* positive control (30193). Both controls are in a liquid bovine serum albumin-based matrix with a non azide preservative. The positive control is a dilution of highly purified *Giardia lamblia* cyst. **Refer to vials for exact concentration range for each control**. After the first use, the controls should be stored at -20°C or below for long-term storage.

7. Concentrated Patient Sample Diluent (Cat. No. 30189)
One bottle contains 30 mL of 20-fold concentrated buffer matrix with protein stabilizers and preservative. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box. Before use the concentrated buffer must be diluted with 570 mL of distilled water and mix well. Upon dilution this yields a working patient sample diluent containing a surfactant in phosphate-buffered saline with a non-azide preservative. The diluted reagent can be stored at room temperature and is stable for 8 weeks. It can also be stored at 2 – 8°C and is stable until the expiration date on the kit box.

SAFETY PRECAUTIONS

The reagents must be used in a laboratory and are for professional use only. Source material for reagents containing bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 50 µL, 100 µL, and 1000 µL, etc.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
5. Disposable plastic 1000 mL bottle with cap.
6. Aluminum foil.
7. Deionized or distilled water.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

SPECIMEN COLLECTION & STORAGE

Fresh fecal sample should be collected by using a plastic sampling device, for example, Epitope Diagnostics Fecal Sample Collection Device (Cat. 30210). It is required to collect a minimum of 0.1 mL liquid stool sample or 0.1 g solid sample. The collected fecal sample must be transported, kept at 2-8°C and tested within 2 days. A non-preserved sample must be stored below -20°C for a longer storage period. Fecal sample collected in 10% formalin or SAF can be stored at 2-25°C for 2 months.

ASSAY PROCEDURE

1. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
(2) Concentrated Patient Sample Diluent (Cat. 30189) must be diluted to working solution prior use. Please see REAGENTS section for details.

2. Patient Sample Preparation

Patient samples need to be diluted 1:11 with patient sample diluent working solution before being measured.

(1) Label a test tube (12x75 mm) or a 1.5 ml plastic vial.
(2) Add 1 mL of assay buffer to each tube or vial.
(3) Add 100 µL of liquid stool sample to the above tube.
(4) With solid stool sample, take an equivalent amount (about 50 – 100 mg) with a spatula or a disposable inoculation loop. Suspend the solid stool sample with 1 mL patient sample diluent and mix well on a vortex mixer.
(5) Centrifuge the diluted fecal sample at 3000 rpm (800-1500 g) for 10 – 15 minutes. The supernatant can be directly used in the assay. As an alternative to centrifuging, let the diluted samples sit and sediment for 15 minutes and take the clear supernatant for testing.
Note: If the test procedure is performed on an automated ELISA system, the supernatant must be particle-free by centrifuging the sample.

3. Assay Procedure

(1) Place a sufficient number of anti-*Giardia* antibody-coated microwell strips (Cat. 30190) in a frame to run giardia controls and unknown samples in duplicate.
(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	Control Negative	SAMPLE 3	SAMPLE 7
B	Control Negative	SAMPLE 3	SAMPLE 7
C	Control Positive	SAMPLE 4	SAMPLE 8
D	Control Positive	SAMPLE 4	SAMPLE 8
E	SAMPLE 1	SAMPLE 5	
F	SAMPLE 1	SAMPLE 5	
G	SAMPLE 2	SAMPLE 6	
H	SAMPLE 2	SAMPLE 6	

(3) Add **100 µL** of controls and diluted patient stool samples into each designated microwell.
(4) Cover the plate with a plate sealer and also with aluminum foil to avoid exposure to light.
(5) Incubate plate at room temperature for **1 hour.**

(6) Prepare working anti-Giardia tracer antibody working solution by **1:21 fold** dilution of the Anti-*Giardia* Tracer Antibody (Cat. 30191) with the Tracer Antibody Diluent (Cat. 30017). For each strip, it is required to mix 1 mL of Tracer Antibody Diluent with 50 µL of Tracer Antibody in a clean test tube.
(7) Remove the plate sealer. Decant the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of deionized or distilled water into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used. **(Note: The plate must not be washed with any ELISA wash buffer!)**
(8) Add **100 µL** of above diluted tracer antibody working solution to each of the wells.
(9) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
(10) Incubate plate at room temperature for **45 minutes**.
(11) Remove the plate sealer. Decant the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of **deionized or distilled water** into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used. ***(Note: The plate must not be washed with any ELISA wash buffer!)***
(12) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
(13) Cover the plate with aluminum foil to avoid exposure to light.
(14) Incubate plate at room temperature for 20 minutes.
(15) Remove the aluminum foil. Add **100 µL** of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
(16) Read the absorbance at 450 nm within 10 minutes in a microplate reader. As an alternative, one can interpret the test results visually by using the color code card included in the kit.

PROCEDURAL NOTES

1. It is recommended that all controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody-coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

INTERPRETATION OF RESULTS

Visual:

1. Positive or reactive: Any sample well that is obviously more yellow than the negative control well.
2. Negative or non-reactive: Any sample well that is not obviously more yellow than the negative control well and the negative color code of the color code card.

Note: The negative control, as well as some patient samples, may show some slight yellow color. A sample well must be obviously darker or more yellow than the negative control well, when it is interpreted as a positive result. Using the color code card provided in the kit would be a help.

ELISA Reader:

1. Calculate the average absorbance for each pair of duplicate test results.
2. Calculate the cut-off
   The positive cut-off and the negative cut-off are established by using following formula.

   Positive Cut-Off = 1.1 x (mean extinction of negative control + 0.10)
   Negative Cut-Off = 0.9 x (mean extinction of negative control + 0.10)

3. Interpret test result
   - **Positive:** patient sample extinction is greater than the Positive Cut-Off
   - **Negative:** patient sample extinction is less than the Negative Cut-Off
   - **Equivocal**: patient sample extinction is between the Positive Cut-Off and the Negative Cut-Off.

4. Assay quality control
   - Positive control must show an average OD reading greater than 0.5.
   - Negative control should show an average OD reading less than 0.20.

EXAMPLE DATA AND CALCULATED CUT-OFF

A typical absorbance data and the resulting negative control and positive control are represented. **This absorbance must not be used in lieu of control values run with each assay.**

	OD 450 nm	Average OD 450 nm
Negative Control	**0.121** **0.117**	**0.119**
Positive Control	**1.163** **1.111**	**1.137**

Positive Cut-Off = 1.1 x (0.119 + 0.10) = 0.241
Negative Cut-Off = 0.9 x (0.119 + 0.10) = 0.197

EXPECTED VALUES

Stool samples from 26 negative specimens and 12 positive specimens are tested with this ELISA.

Samples / Epitope's ELISA	True Positive	True Negative	Total
Positive	10	1	10
Negative	2	25	28
Total	12	26	38

Sensitivity: 83.3% (10/12 = 83.3%)
Specificity: 96.2% (25/26 = 96.2%)
Accuracy: 92.1% (35/38 = 92.1%)

The test results are compared to another commercial fecal Giardia lamblia ELISA, which was tested side-by-side using the same specimens. It also showed the two false negative results from these patient samples.

LIMITATION OF THE PROCEDURE

(1) The results obtained with this Fecal Giardia lamblia antigen test kit serve only as a useful aid to diagnosis. However, the test results should not be interpreted as diagnostic in themselves.
(2) Bacterial or fungal contamination of stool specimens or reagents, or cross-contamination between reagents may cause erroneous results.
(3) Water deionized with polyester resins may deactivate the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay must include both negative and positive controls. For a valid test, the positive control must have an absorbance of at least 0.5 OD units and the negative control must be less than 0.2 OD units. We also recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of this fecal Giardia lamblia antigen ELISA is about 5 ng/mL of Giardia lamblia antigen as determined by testing a series of dilutions of a highly purified sample of Gliadin lamblia antigen with assay buffer and the OD reading is above the positive cut-off.

Reproducibility

The reproducibility of this assay is validated by measuring four samples (two negative and two positive) both in a single assay of 12 replicate determinations and in 6 different assays run on different dates. The results showed a consistent test results interpretation for all the samples.

Specificity

The assay does not cross-react to following organisms: *Cryptosporidium parvum, Rotavirus, Adenovirus*.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state

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This product is developed and manufactured by

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Manufacturer	No. of tests
REF Catalog Number	Keep away from heat and direct sun light
CONC Concentrate	Store at
Read instructions before use	Use by
	LOT Lot No.

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