EPITOPE DIAGNOSTICS, INC.

EDI™ Human Anti-Giardia *lamblia* IgA ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Detection of Human Anti-Giardia lamblia IgA Antibody in serum or plasma

REF KT-845 CE IVD

INTENDED USE

This microplate-based ELISA (enzyme linked immunosorbent assay) kit is intended for the quantitative detection of anti-Giardia lamblia IgA antibody in test sample. The assay is a useful tool in the aid of determination of Giardia lamblia infection in acute or chronic gastroenteritis. This kit is for in vitro diagnostic use only.

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 trIgAer 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 diarrheas. 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* cycts. Generally, symptoms of giardiasis begin 1 to 2 weeks after becoming infected and they may last 2 to 6 weeks.

Despite the fact that Giardia is essentially a luminal pathogen in the gut it does evoke both systemic and local immune responses. Current between serum and secretory antibody responses remains unclear; the presence of anti-Giardia antibodies in serum would be in any way indicative of the development of protective immunity. Evidence emphasizes the importance of secretory antibody for clearance of the pathogen, although other cell-mediated effector mechanisms are also likely to be involved.

Recent studies have found that about 86% of infected patients develop serum antibody (IgA and IgG) against Giardia lamblia. Determination of human anti-giardia antibody may contribute to the aid of clinical diagnosis and understand the status of immune response for each individual.

ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the quantitative measurement of human anti-*Giardia lamblia* IgA in test specimen. The assay utilizes the microplate-based enzyme immunoassay technique by coating highly purified and inactive Giardia *lamblia* antigen onto the wall of microtiter well.

Assay calibrators, controls and unknown specimen are added to microtiter wells of microplate that was coated with a highly purified *Giardia lamblia* antigen on its wall. The *Giardia lamblia* antigen will be bound to the antibody in the liquid calibrators, controls and test samples. The unbound matrices are washed away and a HRP-conjugated antibody which specifically recognizes the specific subtype of human antibody (IgA) is added for further immunoreactions. After an incubation period, an immunocomplex of "*Giardia lamblia* Antigen – human Anti-Giardia IgA – HRP-conjugated Anti-*hIgA* Antibody" is formed if *the human anti-Giardia IgA* 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 the wall of each microtiter well is directly proportional to the amount of human Anti-*Giardia lamblia* IgA 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. Reagents from different kit lot numbers should not be combined or interchanged.

1. Giardia Antigen Coated Microplate (30299)

Microplate coated with highly purified inactive Giardia antigen.

Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to Use.

2.Anti-hIgA Tracer Antibody (30325)

HRP-conjugated anti-human IgA tracer antibody in a stabilized protein matrix.

Qty: 1 x 0.6 mL
Storage: 2 – 8°C
Preparation: 21X Concentrate. The contents must be diluted with tracer antibody diluent (30052) and mixed well before use.

3. Tracer Antibody Diluent (30052)

Buffer for tracer antibody dilution according to the assay procedures.

Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

4. ELISA HRP Substrate (10020)
Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use

5. ELISA Stop Solution (10030)
0.5 M sulfuric acid
Qty: 1 x 12 mL
Storage: 2 – 25°C
Preparation: Ready to Use

6. Giardia IgA Calibrators Levels 1 to 5 (30301-30305)
Giardia IgA antibody in a liquid bovine serum albumin-based matrix with a non-azide preservative.
Qty: 5 x Vials
Storage: After the first use, the calibrators should be stored at -20°C or below for long term storage.
Do not exceed 3 freeze-thaw cycles.
Preparation: Ready to use.

7. Giardia IgA Controls (30306, 30307)
Giardia IgA antibody in a liquid bovine serum albumin-based matrix with a non-azide preservative.
Qty: 2 x Vials
Storage: After the first use, the calibrators should be stored at -20°C or below for long term storage.
Do not exceed 3 freeze-thaw cycles.
Preparation: Ready to use.

8. Assay Buffer Concentrate (10011)
Surfactant in a phosphate buffered saline with non-azide preservative.
Qty: 1 x 30 mL
Storage: 2 – 8°C
Preparation: 10X Concentrate. The contents must be diluted with 270 mL distilled water and mixed well before use.

9. ELISA Wash Concentrate (10010)
Surfactant in a phosphate buffered saline with non-azide preservative.
Qty: 1 x 30 mL
Storage: 2 – 25°C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

SAFETY PRECAUTIONS

The reagents are for in vitro diagnostic use only. Source material which contains reagents of 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 were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. 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 caps.
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

Only 10 µL of human serum (or plasma) is required for Human Anti-giardia IgA measurement. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum samples should be stored at 2 – 8°C up to 48 hours and at –20°C or below for long term storage until measurement. Avoid more than 3x freeze and thaw cycles.

ASSAY PROCEDURE

1. Reagent Preparation

1. Prior to use allow all reagents to come to room temperature (20-25 ºC). Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate (10010) must be diluted to working solution prior use. Please see REAGENTS section for details.

2. Specimen Preparation

1. Samples need to be diluted 1:100 with assay buffer (10011) before being measured.
2. Label a test tube (12 x 75 mm) or a 1.5 mL plastic vial.
3. Add 1 mL of diluted 1X assay buffer to each tube or vial.
4. Add 10 µL of serum or plasma sample to the above tube.

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 microwell strips (30299) in a holder to run calibrators (30301- 30305), controls (30306, 30307), and diluted samples in duplicate.
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
B	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
C	Calibrator Level 2	Control 1	SAMPLE 3
D	Calibrator Level 2	Control 1	SAMPLE 3
E	Calibrator Level 3	Control 2	SAMPLE 4
F	Calibrator Level 3	Control 2	SAMPLE 4
G	Calibrator Level 4	SAMPLE 1	SAMPLE 5
H	Calibrator Level 4	SAMPLE 1	SAMPLE 5

3. Add **100 µL** of calibrators (30301 - 30301), controls (30306, 30307), and diluted samples into the designated microwells. Mix by gently tapping the plate.

4. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **60 minutes.**
5. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
6. Prepare the antibody working solution by 1:21 fold dilution of the tracer antibody (30325) with the diluent (30052). For each strip, it is required to mix 1 mL of the tracer antibody diluent with 50 µL of the tracer antibody in a clean test tube. *Note: This antibody working solution should be freshly prepared.*
7. Add **100 µL** of *antibody working solution* to each well. Mix by gently tapping the plate.
8. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **30 minutes.**
9. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
10. Add **100 µL** of ELISA HRP Substrate (10020) into each of the wells. Mix by gently tapping the plate.
11. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **10 minutes.**
12. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (10030) into each of the wells. Mix by gently tapping the plate.
13. Read the absorbance at **450** within **10 minutes** with a microplate reader.

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 use. Avoid foaming.

INTERPRETION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the calibrator 1 (0 U/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibrator curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.
4. The Giardia IgA concentrations for the controls and samples are read directly from the calibrator curve using their respective corrected absorbance. If log-log graphic paper or computer assisted data reduction program utilizing logarithmic transformation are used, sample having corrected absorbance between the 3.1 U/mL calibrator and the next highest calibrator should be calculated by the formula:

$$\text{Value of unknown} = \frac{\text{Corrected absorbance (unknown)}}{\text{Corrected Absorbance (2nd STD)}} \times \text{Value of the 2nd STD}$$

LIMITATIONS OF THE PROCEDURE

1. The results obtained with this Giardia IgA Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
2. Giardia IgA negative results in untreated patients does not rule out giardiasis when associated with high levels of Giardia IgG antibodies. The finding can often be explained by selective IgA deficiencies, etc.
3. Since there is no Gold Standard concentration available for Giardia IgA measurement, the values of assay calibrators were established and calibrated in arbitrary units (U/mL).
4. For unknown sample value read directly from the assay that is greater than 100 U/mL, it is recommended to measure a further diluted sample for more accurate measurement.
5. Bacterial or fungal contamination of serum specimens or reagents, or cross contamination between reagents may cause erroneous results.
6. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known Giardia IgA levels. We recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

EXPECTED VALUES

Serum from 46 normal adults were measured with this EIA. The following is a guide to interpretation of results. Because the prevalence of human anti-Giardia IgA antibodies may vary depending on a number of factors such as age, gender, geographical location, race, type of test used and clinical history of individual patients, it is strongly recommend that each laboratory should establish its own "normal" range based on populations encountered.

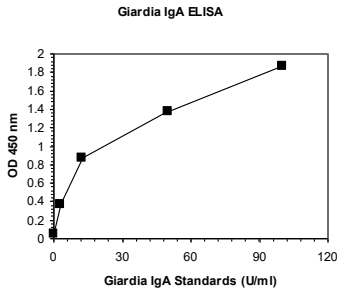
Unit Value	Interpretation
< 5 U/mL	Negative
5 – 10 U/mL	Borderline
> 10 U/mL	Positive

EXAMPLE DATA

A typical absorbance data and the resulting calibration curve are represented.

Note: This curve should not be used in lieu of calibrator curve run with each assay.

Well ID	Reading Absorbance (450 nm)			Concentration (U/mL)
	Readings	Average	Corrected	
Calibrator Level 1: 0 U/mL	0.050	0.050	0.000	
	0.050			
Calibrator Level 2: 3.1 U/mL	0.362	0.364	0.314	
	0.366			
Calibrator Level 3: 12.5 U/mL	0.785	0.784	0.734	
	0.783			
Calibrator Level 4: 50 U/mL	1.371	1.370	1.320	
	1.369			
Calibrator Level 5: 100 U/mL	1.861	1.868	1.818	
	1.875			
Control 1	0.510	0.520	0.470	8.00
	0.530			
Control 2	1.120	1.127	1.077	46.80
	1.134			



PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of this Giardia IgA ELISA as determined by the 95% is minimum of 1 U/mL.

Reproducibility and Precision

The intra-assay precision is validated by measuring two samples in a single assay with 12-replicate determinations. The inter-assay precision is validated by measuring two samples in duplicate in 12 individual assays. The results are as follows:

	Intra-Assay		Inter-Assay	
Sample	**1**	**2**	**1**	**2**
Mean (U/mL)	8.1	46.5	8.5	47.1
CV (%)	5.7	3.2	8.6	7.2

Specificity

This assay does not detect human Anti-Giardia IgG or IgM, as well as other autoantibodies.

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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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

This product is developed and manufactured by

Epitope Diagnostics, Inc.
7110 Carroll Road
San Diego, CA 92121, US

Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

EC	REP

MDSS GmbH
Schiffgraben 41,
30175 Hannover, Germany

GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

IVD In Vitro Diagnostic Device	RUO For Research Use Only	LOT Lot Number
REF Catalog Number	Read instructions before use	Number of Tests
Store at	Use by	Keep away from heat and direct sun light
Manufacturer	EC REP Authorized Representative in Europe	European Conformity

SHORT ASSAY PROCEDURE

1. Add **100 µL** of the calibrators, controls, and diluted samples into the designated microwells.
2. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **60 minutes**.
3. Wash each well five times.
4. Add **100 µL** of the working tracer antibody to each well.
5. Cover and incubate at **room temperature (20-25 ºC)** for **30 minutes**.
6. Wash each well five times
7. Add **100 µL** of substrate to each well.
8. Cover and incubate at **room temperature (20-25 ºC)** for **10 minutes**.
9. Add **100 µL** of the stop solution to each well.
10. Read the absorbance at **450 nm**.