Package Insert

Human Anti-Giardia Iamblia IgG ELISA Assay Kit

Catalog Number:

GIG31-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 7.0 (03.29.23)

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INTENDED USE

Eagle Biosciences Human Anti-Giardia Iamblia IgG ELISA (enzyme linked immunosorbent assay) Assay Kit is intended for the qualitative detection of anti-Giardia lamblia IgG antibody in test sample. The assay is a useful tool in the aid of determination of Giardia lamblia infection in acute or chronic gastroenteritis. The Eagle Biosciences Human Anti-Giardia Iamblia IgG ELISA Assay kit is intended for research use only and not intended for diagnostic procedures.

ASSAY BACKGROUND

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 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 cysts. 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 antibodies (IgA and IgG) against Giardia lamblia. Determination of human anti-giardia antibody may contribute to the aid of clinical diagnosis and understanding of the status of immune response for each individual.

PRINCIPLE OF THE ASSAY

This Eagle Biosciences Human Anti-Giardia Iamblia IgG ELISA Assay Kit is designed, developed and produced for the quantitative measurement of human anti-Giardia lamblia IgG 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 standards, 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 standards, controls and test samples. The unbound matrices are washed away and a HRP-conjugated antibody which specifically recognizes the specific subtype of human antibody (IgG) is added for further immunoreactions. After an incubation period, an immunocomplex of "Giardia lamblia Antigen – human Anti-Giardia IgG-HRP-conjugated Anti-hIgG Antibody" is formed if the human anti-Giardia IgG 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 IgG 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

Microplate coated with highly purified inactive Giardia antigen.

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

2. Anti-hIgG Tracer Antibody

HRP-conjugated anti-human IgG 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 and mixed well before use.

3. Tracer Antibody Diluent

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

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

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

5. ELISA Stop Solution

0.5 M sulfuric acid

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

6. Giardia IgG Calibrators Levels 1 to 5

Giardia IgG 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 IgG Controls

Giardia IgG 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

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

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 research 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 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

- Precision single channel pipettes capable of delivering 10 µL, 50 µL, 100 µL, and 1000 µL, etc.
- Repeating dispenser suitable for delivering 100 µL.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
- Disposable plastic 1000 mL bottle with cap.
- Aluminum foil.
- Deionized or distilled water.
- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- 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 IgG 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. Regents from different kit lot numbers should not be combined or interchanged.

(2) ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.

2. Patient Sample Preparation

(1) Samples need to be diluted 1:100 with assay buffer before being measured.

(2) Label a test tube (12x75 mm) or a 1.5 ml plastic vial.

(3) Add 1 mL of diluted assay buffer (1x) 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 Giardia antigen-coated microwell strips in a frame.
(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	STD 1	STD 5	SAMPLE 2
B	STD 1	STD 5	SAMPLE 2
C	STD 2	C 1	SAMPLE 3
D	STD 2	C 1	SAMPLE 3
E	STD 3	C 2	SAMPLE 4
F	STD 3	C 2	SAMPLE 5
G	STD 4	SAMPLE 1	
H	STD 4	SAMPLE 1	

(3) Add **100 µL** of calibrators, controls 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 plate at room temperature (20-25ºC) for **1 hour**.
(5) Remove the plate sealer. Aspirate the contents of each well. Was each well 5 times by dispensing 350 uL of diluted wash solution into each well, then completely aspirate 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 with the diluent . For each strip, it is required to mix 1 mL of 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 uL 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 (20-25ºC) 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 into each well, then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
(10) Add **100 µL** of ELISA HRP Substrate into each of the wells. Mix by gently tapping the plate.
(11) Cover the plate with a new plate sealer and also with aluminum foil. Incubate at room temperature (20-25ºC) for **15 minutes.**
(12) Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution into each of the wells. Mix by gently tapping the plate
(13) Read the absorbance at 450 nm 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 to use. Avoid foaming.

INTERPRETATION 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 IgG concentration 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

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

LIMITATIONS OF THE PROCEDURE

(1) The results obtained with this Giardia IgG Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
(2) Giardia IgG 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 IgG deficiencies, etc.
(3) Since there is no Gold Standard concentration available for Giardia IgG 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 IgG levels. We recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

EXPECTED VALUES

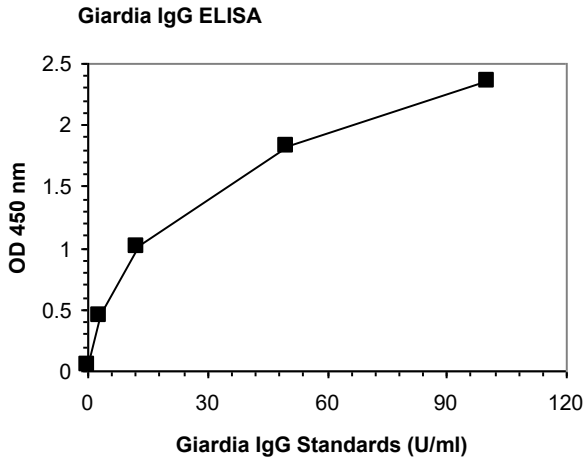
Serum from 46 normal adults were measured with this EIA. The following is a guide to interpretation of results. Because the prevalence of human anti-Giardia IgG antibodies may vary depending on a number of factors such as age, gender, geographical location, race, type of test used and clinical history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

Unit Value	Interpretation
< 10 U/mL	Negative
10 – 16 U/mL	Borderline
> 16 U/mL	Positive

EXAMPLE DATA

A typical absorbance data and the resulting calibrator curve from human anti-Giardia IgG ELISA are represented. **This curve should not be used in lieu of calibrator curve run with each assay.**

Well OD I.D.	450 nm Absorbance			Concentration (U/mL)
	Readings	Average	Corrected	
Calibrator Level 1: 0 U/mL	0.050 0.051	0.051	0.00	
Calibrator Level 2: 3.1 U/mL	0.449 0.445	0.447	0.396	
Calibrator Level 3: 12.5 U/mL	1.001 1.011	1.006	0.955	
Calibrator Level 4: 50 U/mL	1.828 1.820	1.824	1.773	
Calibrator Level 5: 100 U/mL	2.348 2.354	2.351	2.300	
Control 1	0.610 0.614	0.612	0.561	6.97
Control 2	1.215 1.196	1.206	1.155	31.66



PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of this Eagle Biosciences Giardia IgG ELISA Assay Kit as determined by the 95% is of minimum 1 U/mL.

Reproducibility and Precision

The intra-assay precision is validated by measuring two samples in a single assay with 12 replicate determinations.

Mean Giardia IgG Value (U/ml)	CV (%)
7.1	6.2
32.5	4.6

The inter-assay precision is validated by measuring two samples in duplicate in 12 individual assays.

Mean Giardia IgG Value (U/ml)	CV (%)
7.5	8.2
33.1	7.5

Specificity

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

REFERENCES

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4: Wittner M, Maayan S, Farrer W, Tanowitz HB.Diagnosis of giardiasis by two methods. Immunofluorescence and enzyme-linked immunosorbent assay. Arch Pathol Lab Med. 1983 Oct;107(10):524-7.
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Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.