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EDI™ Human Anti-Giardia lamblia IgM ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Detection of Human Anti-Giardia lamblia IgM Antibody



KT 847

EU:



INTENDED USE

This microplate-based ELISA (enzyme linked immunosorbent assay) kit is intended for the qualitative detection of anti-*Giardia lamblia* IgM antibody in test sample. The assay is a useful tool in the aid of determination of *Giardia lamblia* infection in acute or chronic gastroenteritis. It is for professional 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 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 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 infected individual.

ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the quantitative measurement of human anti-*Giardia lamblia* IgM 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 plate.

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 (IgM) is added for further immunoreactions. After an incubation period, an immunocomplex of "*Giardia lamblia* Antigen – human Anti-*Giardia* IgM – HRP-conjugated Anti-*hIgM* Antibody" is formed if the human anti-*Giardia IgM* 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* IgM 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 (Cat. No. 30299)

One microplate with 12 x eight strips (96 wells total) coated with highly purified and inactive *Giardia* antigen. 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-*hIgM* Tracer Antibody (Cat. No. 30300)

One vial containing 0.6 mL concentrated horseradish peroxidase (HRP)-conjugated anti-human IgM tracer 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. 30052)

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 IgM Calibrators (Cat. No. 30331 – 30335)

Five vials each contain Giardia IgM antibody in a liquid bovine serum albumin-based matrix with a non-azide preservative. **Refer to vials for exact concentration for each calibrator.** After the first use, the calibrators should be stored at -20°C or below for long-term storage.

7. Giardia IgM Controls (Cat. No. 30336 – 30337)

Two vials each contain Giardia IgM antibody in a liquid bovine serum albumin-based matrix with a non-azide preservative. **Refer to vials for exact concentration range for each control.** After the first use, the calibrators should be stored at -20°C or below for long-term storage.

8. Assay Buffer Concentrate (Cat. No. 10011)

One bottles each contains 30 mL (10x) concentrated phosphate buffer with protein stabilizers and preservative. The reagent needs to be diluted 1:10 with DI- or DT-water before to use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

9. ELISA Wash Concentrate (Cat. No. 10010)

One bottle contains 30 mL of 30 fold concentrate. Before use the contents must be diluted with 870 mL of distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate-buffered saline with a non-azide preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiration date on the kit box.

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

Only 10 µL of human serum (or plasma) is required for Human Anti-giardia IgM 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.

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 Assay Buffer Concentrate (Cat. 10011) must be diluted to working solution prior use. Please see REAGENTS section for details.
- (3) ELISA Wash Concentrate (Cat. 10010) must be diluted to working solution prior use. Please see REAGENTS section for details.

2. Patient Sample Preparation

Patient samples need to be diluted 1:100 with 1x Assay Buffer Concentrate working concentration before being measured.

- (1) Label a test tube (12x75 mm) or a 1.5 ml plastic vial.
- (2) Add 1 mL of assay buffer (1x) to each tube or vial.
- (3) 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 (Cat. 30299) 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 standards (Cat. 30331-30335), controls (Cat. 30336-30337) and diluted patient serum samples into the designated microwell.
- (4) Cover the plate with one plate sealer.
- (5) Incubate plate at room temperature for **1 hour**.
- (6) Prepare working anti-hIgM Tracer Antibody Working Solution by **1:21 fold** dilution of the tracer antibody (Cat. 30300) with the Tracer Antibody Diluent (Cat. 30052). For each strip, it is required to mix 1 mL of Tracer Antibody Diluent with 50 µL of IgM Tracer Antibody in a clean test tube.

SAFETY PRECAUTIONS

The reagents must be used in a laboratory and is for professional use only. The source of 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.

- (7) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
- (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 **30 minutes**.
- (11) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
- (12) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
- (13) Cover the plate with a new plate sealer and also with aluminum foil to avoid exposure to light.
- (14) Incubate plate at room temperature for **15 minutes**
- (15) Remove the aluminum foil and plate sealer. 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.

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.

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.

The Giardia IgM 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:

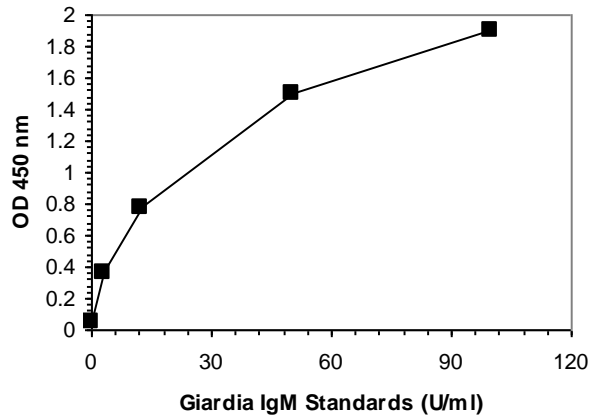
$$\text{Value of unknown} = \frac{\text{Corrected absorbance (unknown)}}{\text{Corrected Absorbance (2}^{\text{nd}} \text{ STD)}} \times \text{Value of the 2}^{\text{nd}} \text{ STD}$$

EXAMPLE DATA AND CALIBRATOR CURVE

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

| Well I.D. | OD 450 nm Absorbance | | | Results ng/mL |
|-----------|----------------------|---------|-----------|---------------|
| | Readings | Average | Corrected | |
| 0 | 0.045 | 0.045 | 0.000 | |
| U/mL | 0.044 | | | |
| 3.1 | 0.361 | 0.368 | 0.323 | |
| U/mL | 0.375 | | | |
| 12.5 | 0.785 | 0.770 | 0.725 | |
| U/mL | 0.755 | | | |
| 50 | 1.493 | 1.498 | 1.453 | |
| U/mL | 1.503 | | | |
| 100 | 1.896 | 1.897 | 1.852 | |
| U/mL | 1.898 | | | |
| Control 1 | 0.512 | 0.518 | 0.473 | 8.16 |
| | 0.523 | | | U/mL |
| Control 2 | 1.241 | 1.239 | 1.194 | 41.09 |
| | 1.237 | | | U/mL |

Giardia IgM ELISA



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 IgM 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 |
|--------------|----------------|
| < 25 U/mL | Negative |
| 25 – 30 U/mL | Borderline |
| > 30 U/mL | Positive |

LIMITATION OF THE PROCEDURE

- (1) The results obtained with this Giardia IgM Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
- (2) Giardia IgM 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 IgM deficiencies, etc.
- (3) Since there is no Gold Standard concentration available for Giardia IgM 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 inactivate the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known Giardia IgM levels. We 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 Giardia IgM ELISA as determined by the 95% is of minimum 1 U/mL.

Precision

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

| Mean Giardia IgM Value (U/mL) | CV (%) |
|-------------------------------|--------|
| 8.26 | 6.2 |
| 42.12 | 4.5 |

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

| Mean Giardia IgM Value (U/mL) | CV (%) |
|-------------------------------|--------|
| 8.31 | 7.6 |
| 41.89 | 6.1 |

Specificity

This assay does not detect human Anti-Giardia IgA or IgG, 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. Epitepe Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitepe 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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|-------------------------------------|--|
| Manufacturer | No. of tests |
| Catalog Number | Keep away from heat and direct sun light |
| Concentrate | Store at |
| In Vitro Diagnostic Device | Use by |
| Read instructions before use | Lot No. |
| Authorized Representative In Europe | |

