Human Strongyloides IgG/IgM ELISA Kit

Enzyme Immunoassay for the determination of Human Strongyloides IgG / IgM in serum and plasma (citrate, heparin).

Catalog number: ARG82310

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Strongyloides (from Greek strongylos, round, + eidos, resemblance), anguillula, or threadworm is a genus of small nematode parasites, belonging to the family Strongylidae, commonly found in the small intestine of mammals (particularly ruminants), that are characterized by an unusual lifecycle that involves one or several generations of free-living adult worms.

Human infection, strongyloidiasis, is chiefly caused by *Strongyloides stercoralis*, widespread in all tropical regions, or by *Strongyloides fuelleborni*, a parasite of primates in African and Asian tropics and of humans in African tropics and New Guinea. Other species include *Strongyloides papillosus* found in cattle, pigs, sheep, goats, rabbits, and rats, *Strongyloides ransomi* found in pigs, and *Strongyloides ratti*, found in rats.

Treatment for *strongyloides* infection is ivermectin or thiabendazole. The presence of pathogen or infection may be identified by microscopy or serology method like ELISA. [Provide by Wikipedia: *Strongyloides*]

PRINCIPLE OF THE ASSAY

This assay employs the enzyme-linked immunosorbent technique. The specific antigens (strongyloides antigens) has been pre-coated onto a microtiter plate. The diluted samples or Controls are added to the pre-coated ELISA plate. After incubation, the wells are washed with diluted Wash Buffer to remove unbound material. Then Strongyloides Conjugate is added and incubated. After incubation, the wells are washed with Wash Buffer to remove unbound material. Then the TMB substrate is added to the wells and color develops in proportion to the amount of specific antibody binding in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. And run validation by Substrate Blank, Negative Control and Positive Control to calculate the Units (U) of samples.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Strongyloides Binding microplate	8 X 12 strips	4°C
Positive Control (coloured yellow)	2 mL	4°C
Cut-off Control (coloured yellow)	3 mL	4°C
Negative Control (coloured yellow)	2 mL	4°C
Diluent Buffer (coloured yellow)	100 mL (ready to use)	4°C
Strongyloides Conjugate (HRP-Strongyloides, coloured blue)	20 mL (ready to use)	4°C
20X Wash Buffer	50 mL	4°C
TMB substrate	15 mL (ready to use)	4°C (protect from light)
STOP solution	15 mL (ready to use)	4°C
Cover foil	1 ea	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Incubator (37°C)
- Votex mixer
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- The remaining strips should be immediately resealed in the aluminum foil along with the desiccant supplied and stored at 2-8 °C.
- Briefly spin down the all vials before use.
- It is important to bring all reagents and samples to room temperature (20-25°C) and mix them before starting the test run.
- If crystals are observed in the 20X Wash buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.

- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, do NOT use it.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Collect the plasma layer and store on ice.

Sample Dilution: Before assaying, all samples should be diluted 1+100 with Diluent Buffer. Dispense 10 µL sample and 1 mL Diluent Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a vortex mixer.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Do not use heat inactivation specimens.
3. Avoid disturbing the white buffy layer when collection serum/plasma sample.
4. Aliquot samples for testing and store at -80°C. Avoid repeated freeze-thaw cycles. Perform dilutions in Diluent buffer as necessary.
5. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 20X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 25 mL of 20X Wash Buffer into 475 mL of distilled water to a final volume of 500 mL) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Each diluted sample and Controls should be assayed in duplicate or triplicate. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of 1X Wash Buffer from 300 µL to 350 µL to avoid washing effects.

1. Add 100 µL of the diluted samples and Controls to each well, and leave a well for the Substrate Blank.
2. Cover wells with the foil and incubate at 37°C for 1 hour on a microplate shaker.
3. Remove the foil, aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µL of Strongyloides Conjugate to each well except for the Substrate Blank well. Incubate at RT for 30 minutes on a microplate shaker.

Do not expose to direct sunlight.

5. Aspirate each well and **wash as step 3**.
6. Warm **TMB Substrate** to **RT**. Add **100 µL** of **TMB Substrate** to each well, including the Substrate Blank wells. Incubate for exactly **15 minutes** at **RT** in the dark.
7. Add **100 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at **450nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance **within 30 minutes** after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Substrate Blank, Controls and diluted samples.
2. Adjust the ELISA microwell plate reader to zero using the Substrate Blank. If- due to technical reasons- the ELISA microwell plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results.
3. In order for an assay to be considered valid, the following criteria must be met:

   Substrate Blank: Absorbance value **< 0.100**

   Negative Control: Absorbance value **<0.200 and < Cut-off**

   Cut-off Control: Absorbance value **0.150-1.300**

   Positive Control: Absorbance value **> Cut-off**

   If these criteria are not met, the test is not valid and must be repeated.
4. Calculation of Results:

   The **Cut-off** is the mean absorbance value of the Cut-off Control determinations.

   Example: Duplicate of Cut-off Control absorbance value are 0.44, 0.42

   Cut-off = 0.44 + 0.42 / 2 = 0.43

   Results in Units (U)

   Sample absorbance value (mean) X 10 / Cut-off = Units (U)

   Example: 1.591 X 10 / 0.43 = 37 U

5. Interpretation of Results:

Cut-off	10U	
Positive	>11U	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9-11U	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as negative.
Negative	<9U	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV value of intra-assay was 3.89-7.27% and inter-assay was 9.69-10.72%.

Diagnostic specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 94.12% (95% confidence interval: 83.76% - 98.77%).

Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 89.47% (95% confidence interval: 75.2% - 97.06%).