Human Ascaris lumbricoides IgG antibody ELISA Kit

Enzyme Immunoassay for the qualitative determination of IgG class antibodies against Ascaris lumbricoides in human serum or plasma.

Catalog number: ARG80511

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INTRODUCTION

Ascaridae are big nematodes. The male individuals are up to 25 cm, the female ones are up to 40 cm long. Ascaris lumbricoides is among the Ascaridae the species with the highest importance for human medicine, because it is the only one with humans as main host.

The sexually mature roundworm lives in the small intestine. The females produce up to 200 000 eggs daily, which attain to the environment by faeces. Infectious larvae develop inside the eggs and after oral ingestion they hatch in the upper part of the small intestine. They penetrate the wall of the intestine and get into the venous blood with which they get into liver and lung, where they leave the vessels and skin in the aveoles. The larvae migrate into the trachea and through the pharynx after swallowing back to the small intestine where the maturation to the adult worm takes place. Ca. 10-12 weeks after infestation the roundworms will be excreted with faeces. The adult worm lives for around 18 months.

Ascaris lumbricoides is one of the most abundant exciter of infectious diseases worldwide. Main endemic areas are Eastern Asia, Africa and Middle and South America. Children are more often affected than adults. The infestation leads to Ascariasis mostly with latent progression. The migrating larvae can lead to inflammatory, eosinophile infiltration of the lung and cause cough, dyspnoea and light fever. Conglomerates of the worms can cause intestinal blockage. If the worms migrate into gall, pancreas or stomach the corresponding clinical symptoms result.

Species	Disease	Symptoms	Mechanism of Infection
Ascaris lumbricoides	Ascariasis	- Adult worms cause no symptoms in general. - Conglomerates of worms can cause abdominal pain and ileus. - Infection of gall, stomach or pancreas leads to corresponding symptoms. - Migrating larvae are able to cause pumonal symptoms like cough and dyspnoea.	Ingestion of infectious Ascaridae eggs (classical way of infestation is the consumption of head dunged salad)

The presence of an infection may be identified by:

Microscopy: Detection of eggs in faeces

Serology: Detection of antibodies by ELISA

PRINCIPLE OF THE ASSAY

This assay employs the qualitative determination enzyme immunoassay technique. An antigen specific for Ascaris lumbricoides has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any antibody present is bound by the immobilized antigen. After washing away any unbound substances, a Horseradish Peroxidase (HRP) labeled Protein conjugate is added to each microplate well and incubated. This conjugate binds to antigen-antibody complexes. After washing away any unbound reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ascaris lumbricoides IgG bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Antigen-coated microplate	1 plate	4°C.
Control Negative	2 ml (ready for use)	4°C
Control Cut-off	3 ml (ready for use)	4°C
Control Positive	2 ml (ready for use)	4°C
Protein A conjugate	1 vial (20ml) (ready for use)	4°C (Protect from light)
Sample Diluent	100 ml (ready for use)	4°C
20X Wash buffer	50 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using citrate or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay

immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. Diluted wash buffer is stable for 5 days at RT.
- Samples: Samples have to be diluted 1+100 with sample diluent. (e.g. Dispense 10µl sample and 1ml Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.)

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of controls into control wells.
3. Add 100 µl of samples into sample wells.
4. Cover wells and incubate for 60 minutes at 37°C.
5. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (350 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.
6. Add 100 µl of Protein A conjugate into each well. Cover wells and

incubate for 30 minutes at RT.

7. Aspirate each well and wash as step 5.
8. Add 100 µl of TMB mixture to each well. Incubate for 15 minutes at room temperature in dark.
9. Add 100 µl of Stop Solution to each well.
10. Read the OD with a microplate reader at 450/620 nm immediately. It is recommended reading the absorbance within 30 min after adding Stop solution.

MEASUREMENT

1. 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.
2. Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard/control and sample in the plate layout. Bichromatic measurement using a reference wavelength of 620 nm is recommended. Where applicable calculate the mean absorbance values of all duplicates.

RESULTS

1. Run Validation Criteria

   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.

2. Calculation of Results

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

   Absorbance value Cut-off Control (0.44) + absorbance value Cut-off control (0.42) = 0.86 / 2 = 0.43

   Cut-off = 0.43

3. Results in Units [U]

   [Units = U] = (Sample (mean) absorbance value x 10) / Cut-off

   Example: (1.591 x 10) / 0.43 = 37 U

4. Interpretation of Results

   - Positive: > 11 U

     Antibodies against the pathogen are present.

     There has been a contact with the antigen (pathogen resp. vaccine).

   - Equivocal: 9-11 U

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: < 9 U

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 precision was 5.9% and inter-assay precision was 3.45%.

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 95.0 % (95% confidence interval: 87.69% - 98.62%).

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 100.0 % (95% confidence interval: 47.82% - 100.0%).

Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.

Cross Reactivity

Cross reaction with antibodies against Toxocara canis, Trichinella, Fasciola, Filaria and Strongyloides cannot be excluded.