Human Toxocara canis IgG antibody ELISA Kit

Enzyme Immunoassay for the qualitative screening of human IgG antibodies to Toxocara canis in human serum and plasma.

Catalog number: ARG80611

For research use only. Not for use in diagnostic procedures.

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PRINCIPLE OF THE ASSAY

This assay employs the qualitative enzyme immunoassay technique. The Toxocara canis antigens have been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated protein A is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab 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. The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

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	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
IgG Sample Diluent	100 ml (Ready-to-use)	4°C
Control A (negative)	2 ml (Ready-to-use)	4°C
Control B (cut-off)	3 ml (Ready-to-use)	4°C
Control C (positive)	2 ml (Ready-to-use)	4°C

HRP-protein A conjugate	20 ml (Ready-to-use)	4°C
20X Wash buffer	50ml	4°C
TMB substrate	15ml	4°C (Protect from light)
STOP solution	15ml	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.

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 heparin or citrate 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.
- Patient sample: Dilute patient sample 1:101 with Sample diluent buffer before assay, mix well. (e.g. 5 µl of serum + 500 µl of sample diluent buffer)

  Note: the controls are ready-to-use and need not further dilution.

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 (1 well for negative or positive control; 2 wells for the cut-off control) and diluted samples and into wells. Leave one well for substrate blank.
3. Incubate for 60 minutes at 37°C.
4. Aspirate each well and wash, repeating the process once for a total 2 washes. Wash by filling each well with 1× Wash Buffer (350µl) 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.
5. Add 100 µl HRP-protein A conjugate into each well (except blank). Incubate for 30 minutes at RT.
6. Wash as according to step 4.
7. Add 100 µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature in the dark.
8. Add 100 µl of Stop Solution to each well. Incubate for 5 minutes at RT. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

Adjust the ELISA reader to zero using the substrate blank.

In order for an assay to be considered valid, the following criteria must be met:

Substrate blank: Absorbance value < 0.100

Negative: Absorbance value < 0.200 and < OD cut-off

Positive: Absorbance value > OD cut-off

Cut-off: Absorbance value 0.15-1.30

INTERPRETATION OF RESULTS

Sample are considered Positive if the absorbance value is higher than 10% over cut-off.

Grey zone: Sample with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative.

Sample are considered Negative if the absorbance value is lower than 10% below cut-off.

Results in Units:

$$\text{Patient (mean)absorbance value x } 10 / \text{Cut} - \text{off} = [Units = U]$$

Cut-off: 10 U

Grey zone: 9-11

Positive: >11 U

Negative: <9 U

QUALITY ASSURANCE

Interference

Interferences with hemolytic, lipemic or icteric serum are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 2.6% and inter-assay precision was 8.1%.