Version 22-04/24

**User's Manual**

# Toxocara Canis IgG ELISA Kit

REF **DEIA1796**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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# PRODUCT INFORMATION

## Intended Use

For the qualitative screening of serum IgG antibodies to Toxocara using an Enzyme-Linked Immunosorbent Assay (ELISA) technique.

For research use only.

## Principles of Testing

The micro test wells are coated with an excretory/secretory antigen from the Toxocara larvae. During the first incubation with the diluted patients' sera, any antibodies which are reactive with the antigen will bind to the coated wells. After washing to remove the rest of the sample, the Enzyme Conjugate is added. If antibodies have been bound to the wells, the Enzyme Conjugate will then bind to these antibodies. After another series of washes, a chromogen (tetramethylbenzidine or TMB) is added. If the Enzyme Conjugate is present, the peroxidase will catalyze a reaction that consumes the peroxide and turns the chromogen from clear to blue. Addition of the Stop Solution ends the reaction and turns the blue color to a bright yellow color. The reaction may then be read visually or with an ELISA reader.

## Reagents And Materials Provided

| Item | Description | Symbol |
|---|---|---|
| Test Strips | Microwells containing *Toxocara* antigens – 96 test wells in a test strip holder. | MT PLATE |
| Enzyme Conjugate | One (1) bottle containing 11 ml of Protein A conjugated to peroxidase. | CONJ |
| Positive Control | One (1) vial containing 1 ml of diluted positive rabbit serum. | CONTROL + |
| Negative Control | One (1) vial containing 1 ml of diluted negative human serum. | CONTROL - |
| Chromogen | One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB). | SUBS TMB |
| Wash Concentrate (20X) | One (1) bottle containing 25 ml of concentrated buffer and surfactant. | WASH BUF |
| Dilution Buffer | Two (2) bottles containing 30 ml of buffered protein solution. | SPECIM DIL |
| Stop Solution | One (1) bottle containing 11 ml of 0.73 M phosphoric acid. | SOLN |

## Materials Required But Not Supplied

Pipettes

Squeeze bottle for washing strips (narrow tip is recommended)

Reagent grade water and graduated cylinder

Tubes for sample dilution

Absorbent paper

### Suggested Materials

ELISA plate reader with a 450 nm and a 650 to 620 nm filter (optional if results are read visually)

## Storage

Reagents, strips and bottled components:

Store between 2 – 8 °C.

Squeeze bottle containing diluted wash buffer may be stored at room temperature.

## Specimen Collection And Preparation

### Serum

Serological specimens should be collected under aseptic conditions. Coagulate blood and remove serum. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analyzed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipemic and strongly hemolytic serum should be avoided. Do not heat inactivate serum and avoid repeated freezing and thawing of samples.

### Test samples:

Make a **1:64** dilution of patients' sera using the dilution buffer (e.g. 5 µl sera and 315 µl dilution buffer).

## Reagent Preparation

### Wash Buffer

Remove cap and add contents of bottle to 475 ml of reagent grade water. Place diluted wash buffer into a squeeze bottle with a narrow tip opening.

**Note:** Washings consist of filling to the top of each well, shaking out the contents and refilling.

Avoid generating bubbles in the wells during the washing steps.

## Assay Procedure

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Add 100 µl (or two drops) of the negative control to well #1, 100 µl of the positive control to well #2 and 100 µl of the diluted (1:64) test samples to the remaining wells.

**Note:** Negative and positive controls are supplied prediluted. Do not dilute further.

3. Incubate at room temperature (15 to 25 °C) for 10 minutes.
4. Shake out contents and wash 3 times with the diluted wash buffer.
5. Add 2 drops of Enzyme Conjugate to each well.
6. Incubate at room temperature for 5 minutes.
7. Shake out contents and wash 3 times with wash buffer. Slap plates against paper toweling to remove excess moisture.
8. Add 2 drops of the Chromogen to every well.
9. Incubate at room temperature for 5 minutes.
10. Add 2 drops of the Stop Solution and mix by tapping strip holder.

### Reading of Results

Visually:

Look at each well against a white background (e.g. paper towel) and record as clear or +, ++ or +++ reaction.

ELISA Reader:

Zero reader on air. Set for bichromatic readings at 450/650-620 nm.

## Quality Control

The use of controls allows validation of kit stability. The kit should not be used if any of the controls are out of range.

Expected values for the controls are:

**Negative** - 0.0 to 0.3 OD units

**Positive** - 0.5 OD units and above

### Troubleshooting

Negative control has excessive color after development.

**Reason**: inadequate washings.

**Correction**: wash more vigorously. Remove excessive liquid from the wells by tapping against an absorbent towel. Do not allow test wells to dry out.

## Interpretation Of Results

### Interpretation of Results - ELISA Reader

Zero ELISA reader on air. Read all wells at 450/650-620 nm.

**Positive** - Absorbance reading equal to or greater than 0.3 OD units.

**Negative** - Absorbance reading less than 0.3 OD units.

A negative OD reading indicates that the patient has no detectable level of antibodies. This may be due to lack of infection or poor immune response by the patient.

### Interpretation of Results - Visual

Compare results to the controls.

A sample should be interpreted as positive if the degree of color development is significant and obvious.

### Expected Results

The number of individuals showing positive results can vary significantly between populations and geographic regions. If possible, each laboratory should establish an expected range for its patient population.

### Performance Data

Study #1 – Canadian Reference Center

Compared CD ELISA to another commercial ELISA. Found concordance of 84% (n=82).

Study #2 – Mayo Clinic

Sensitivity of 87.5% (21/24)

Specificity of 93.3% (14/15)

| | Reference Method | |
|---|---|---|
| | + | - |
| + | 21 | 1 |
| - | 3 | 14 |

## Detection Limit

Serologic results are an aid in diagnosis but cannot be used as the sole method of diagnosis.

## Precautions

Do not use solutions if they precipitate or become cloudy.

Wash concentrate may show crystallization upon storage at 2 – 8 °C. Crystallization will disappear after dilution to working strength.

Do not use serum that may have supported microbial growth, or is cloudy due to high lipid content. Samples high in lipids should be clarified before use.

Treat all sera as if capable of being infectious. Negative control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods. This product should be used under appropriate safety conditions that would be used for any potentially infectious agent.

Do not add azides to the samples or any of the reagents.

## References

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