S. stercoralis IgG ELISA Kit

Catalog Number KA3203
96 assays
Version: 01

Intended for research use only
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Introduction

Intended Use

The *S. stercoralis* IgG ELISA Kit is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgG antibodies to *Strongyloides stercoralis* in serum and plasma. The *Strongyloides* ELISA test is for use only by a laboratory.

Background

The parasite *Strongyloides stercoralis* is the causative agent for Strongyloidiasis disease. These parasites are endemic to tropical and semi-tropical environs, but are also found in various regions throughout the world. The parasite is known as an intestinal nematode which invades the body through exposed skin. Intestinal problems occur, such as diarrhea, but in more severe cases where the patient's immune system may be compromised, meningitis or septic shock may develop.

The *S. stercoralis* IgG ELISA Kit is an especially appropriate serological test to use if the infection occurs outside the intestine where it is important to keep the disease isolated from other conditions, such as hemotologic malignancies.

Principle of the Assay

The principle of the *S. stercoralis* IgG ELISA Kit is a three-incubation process whereby the first incubation involves the coating of the wells with *Strongyloides* antigen. During this step, any antibodies that are reactive with the antigen, will bind to the coated wells. Next, the wells must be washed to remove test sample. At this point enzyme conjugate is added. During this second incubation, the enzyme conjugate will bind to any antibodies present. Before the third incubation step, more washings are necessary. Then, the chromogen (tetramethylbenzidine or TMB) is added. With the presence of enzyme conjugate and the peroxidase causing the consumption of peroxide, the chromogen changes to a blue color. The blue color turns to a bright yellow color after the addition of the stop solution, which ends the reaction. ELISA readers can be used to obtain results, or results may be read visually.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>Microwells containing Strongyloides antigens in a test strip holder.</td>
<td>96-wells</td>
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<tr>
<td>Enzyme Conjugate: Protein A conjugated to peroxidase.</td>
<td>11 ml</td>
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<tr>
<td>Negative Control: Diluted negative human serum.</td>
<td>1 ml</td>
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<tr>
<td>Positive Control: Diluted positive rabbit serum.</td>
<td>1 ml</td>
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<tr>
<td>TMB Substrate: Stabilized tetramethylbenzidine (TMB).</td>
<td>11 ml</td>
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<tr>
<td>Wash Buffer Concentrate (20X): Concentrated buffer and surfactant with preservative.</td>
<td>2 bottles x 25 ml</td>
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<tr>
<td>Milk Dilution Buffer: buffered protein solution.</td>
<td>30 ml</td>
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<tr>
<td>Stop solution: 1 M phosphoric acid.</td>
<td>11 ml</td>
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</table>

Storage Instruction

Reagents, strips and bottled components: Store between 2–8 °C.
Squeeze bottle containing diluted wash buffer may be stored at room temperature.

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

Precautions for Use

1. Important note:
✓ 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.
Assay Procedure

Reagent Preparation

- Wash Buffer
  Remove cap and add contents of one bottle to 475 ml DI water. Transfer contents of 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.

Sample Preparation

Coagulate blood and remove serum. Freeze sample at -20 °C or lower if not used immediately.
Do not heat inactivate serum and avoid repeated freezing and thawing of samples.

Test samples: Make a 1:64 dilution of patient's sera using the dilution buffer (e.g. 5 µl sera and 315 µl dilution buffer).

Assay Procedure

All incubations are at room temperature (15 to 25 °C)

1. Break off the required number of wells (number of samples plus 2) and place in strip holder.
2. Add 100 µl of the negative control to well #1 and 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 wells against paper towels 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.
Data Analysis

Interpretation of Results

- **Visual**
  Compare results to the controls. A sample should be interpreted as positive if the degree of color is significant and obvious.
  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.

- **ELISA Reader**
  Zero ELISA reader on air. Read all wells at 450/650-620 nm.
  Positive - Absorbance reading greater than 0.2 OD units.
  Negative - Absorbance reading less than 0.2 OD units.

A positive OD reading indicates that the patient may be infected by Strongyloides.
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.

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

- **Quality Control**
  The Positive and Negative Controls must be run each time the assay is performed.
  For a valid run, the Negative Control must be below 0.15 ODs and the Positive Control greater than 0.5 OD units. If either Control is out of range, do not use the kit and contact Abnova.

  Problem: Negative control has substantial color development.
  Correction: Washings were insufficient. Repeat test with more vigorous washings.

- **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.

- **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.

Performance Characteristics

- Study #1: A total of 28 stools were tested against culture.

The following results were obtained.

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<th>Reference Method</th>
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Specificity: 14/14 = 100%

Sensitivity: 14/14 = 100%
Resources

Reference


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