E. Histolytica IgG ELISA Kit

Catalog Number KA3193
96 assays
Version: 01

Intended for research use only
Introduction

Intended Use

The *E. Histolytica* IgG ELISA Kit is an enzyme linked immunosorbent assay (ELISA) for the qualitative identification of serum IgG antibodies to Entamoeba histolytica.

Background

Generally, the disease of Amebiasis is found in a number of tropical regions where living conditions and poor sanitation cause significant health problems. Transmission of the disease centers around native populations and tourists traveling from these areas. A protozoan parasite called Entamoeba histolytica is the causing agent of Amebiasis, and the disease usually shows up as intestinal problems. Symptoms are generally mild, but in some cases the organism becomes extra-intestinal and can lead to abscesses, primarily affecting the liver. Serological tests are recommended for extra-intestinal diagnosis, making sure to isolate the disease from other diseases of the liver, or ulcerative colitis, for example. This *E. Histolytica* (Amebiasis) ELISA Test should not be used for diagnosing intestinal infections. Intestinal infections are conventionally established through an Ova and Parasite (O&P) test, or an *E. histolytic* fecal antigen assay. A positive result may not automatically be evidence of an active infection, and a negative outcome at least assures exclusion of a suspected *E. histolytica* tissue invasion.

Principle of the Assay

The principle of the *E. Histolytica* IgG ELISA Kit is a three-incubation process whereby the first incubation involves the coating of the wells with *E. histolytic* antigen. During this step, any antibodies that are reactive with the antigen, will bind to the 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 a 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 the results can be read visually.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Test Strips: Microwells containing E. histolytica strain NIH-200 antigens</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>Positive Control: diluted positive rabbit serum</td>
<td>1 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>TMB substrate Solution: chromogen tetramethylbenzidine (TMB).</td>
<td>11 ml</td>
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<tr>
<td>Wash Concentrate (20X): concentrated buffer and surfactant</td>
<td>25 ml</td>
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<tr>
<td>1 % Dilution Buffer: buffered protein solution</td>
<td>30 ml</td>
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<tr>
<td>Stop Solution: 0.73 M phosphoric acid</td>
<td>11 ml</td>
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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 450 and 620-650 nm filters

Precautions for Use

- 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.

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

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.*

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 patients' sera using the dilution buffer (e.g. 5 µl sera and 315 µl dilution buffer).

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 ºC) 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

Calculation of Results

- 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

- Interpretation of Results - ELISA Reader
  Zero ELISA reader on air. Read all wells at 450/650-620 nm.
  Positive: Absorbence reading greater than 0.4 OD units.
  Negative: Absorbence reading less than 0.4 OD units.
  A positive OD reading indicates that the patient may be infected by E. histolytica.
  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 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 Characteristics

Study #1- Canadian Reference Center
  Compared DAI ELISA to another commercial ELISA. Found concordance of 96.3% (n=82).
### Study #2 - CDC&P

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<th>CDC&amp;P</th>
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<tr>
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<td>+</td>
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<tr>
<td>Abnova</td>
<td>+</td>
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Sensitivity of 92% (22/24)
Specificity of 100% (21/21)
Resources

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.

Reference


