Tetanus toxoid Ab ELISA Kit

Catalog Number KA3298

96 assays

Version: 01

Intended for research use only
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Introduction

Intended Use

The Tetanus toxoid Ab ELISA Kit is an enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of antibodies to tetanus toxoid in human serum.

Background

Tetanus toxins are some of the most dangerous poisons in the world and can affect individuals of all ages. Tetanus is a disease caused by the bacterium Clostridium tetani. The toxin can be found in a variety of places, but most typically in the environment through animal waste or in soil. An individual is commonly infected through a skin wound, and then the bacteria travels throughout the body causing damage to muscles and the nervous system. If not treated, symptoms can develop into severe muscle spasms.

Tetanus toxoid Ab ELISA kits have been found to be some of the most effective diagnostic tools to determine tetanus antitoxins in human serum. Because of their sensitivity and rapid test results, these ELISA kits have prevailed over more conventional methods for detecting antibodies to tetanus toxoids.

Principle of the Assay

The principle of the Tetanus toxoid Ab ELISA test is a three-incubation process whereby the first incubation involves the coating of the wells with tetanus toxoid antigen. During this step with the diluted patients' sera, 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 washing 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 reaction can be ready visually.
General Information

Materials Supplied

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<tr>
<th>Component</th>
<th>Amount</th>
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<tr>
<td>Test Strips: Microwells containing tetanus toxoid antigens - 96 test wells in a test strip holder.</td>
<td>96 wells</td>
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<td>Enzyme Conjugate: One bottle containing Protein A conjugated to peroxidase.</td>
<td>11 ml</td>
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<td>Standards: Four vials containing diluted positive human serum.</td>
<td>4x1 ml</td>
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<td>TMB Substrate Solution: One bottle containing the chromogen tetramethyl-benzidine (TMB).</td>
<td>11 ml</td>
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<td>Wash Concentrate (20X): One (1) bottle containing 25 ml of concentrated buffer and surfactant.</td>
<td>25 ml</td>
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<td>1% Dilution Buffer: Two bottles containing buffered protein solution.</td>
<td>2x30 ml</td>
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<td>Stop Solution: One bottle containing 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 ºC. 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

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 ºC. 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. Standards have 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.

- Test Limitations
  This assay determines the relative amount of anti-tetanus antibodies in serum. It cannot be used to diagnose active disease or conclusively determine immune/non-immune status.
Assay Protocol

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 1:100 and a 1:1,000 dilution of patient's sera using the dilution buffer.

Assay Procedure

1. Break off number of wells needed (four for calibrators plus number of samples) and place in strip holder.
2. Add 100 µl of each calibrator to wells 1-4, then 100 µl of the diluted test samples to the remaining wells. Note: Standards 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, then rinse once with DI water. Slap wells against paper towels to remove excess moisture.
8. Add 2 drops of the Chromogen to every well. Mix by gently tapping strip holder.
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
ELISA Reader: Zero reader on air. Set for bichromatic readings at 450/650-620 nm.

• Interpretation of Results
Construct a standard curve using the absorbence (OD) results of the four controls and the controls’ International Units (IU) included in the kit. All graphs should be on log-log 10 paper: Y axis for absorbence and X axis for IU's. Plot the control coordinates and determine the best-fit line. Using the absorbence data and the standard curve as a guide, determine the approximate IU for each sample. Once the IU value has been determined by the graph, multiply this number by the dilution factor of the sample.

Example:
Sample "A" has an absorbance of 0.4 OD units at a 1:100 serum dilution. This OD value corresponds to an IU value of 0.008 IU/ml. Thus the sample has a value of 0.8 IU/ml (0.008 x 100 dilution).
Resources

Troubleshooting

Negative control has excessive color after development.
✓ Reason: inadequate washings.
✓ Correction: repeat test with more vigorous washings. Remove excessive liquid from the wells by tapping against an absorbent towel. Do not allow test wells to dry out.

References

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