CE

AccuDiag™ Tetanus IgG ELISA Kit

Cat # 8205-35

IVD See external Label 8°C 2°C 96 Tests

Test	Tetanus IgG ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	Quantitative: Positive, Negative
Sample	5 µL serum
Total Time	~ 20 min.
Shelf Life	12 Months from the manufacturing date
Specificity	Not Determined
Sensitivity	Not Determined

INTENDED USE

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

SUMMARY AND EXPLANATION

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 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.

TEST PRINCIPLE

The principle of the Tetanus 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 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 reaction can be ready visually.

SPECIMEN COLLECTION AND PREPARATION

Coagulate blood and remove serum. Freeze sample at -20 ºC 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.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Plate:** Microwells containing Tetanus toxoid antigens - 96 test wells in a test strip holder.
2. **Enzyme Conjugate**: One (1) bottle containing 11 ml of Protein A conjugated to peroxidase.
3. **Standards:** Four (4) vials containing 1 ml of diluted positive human serum.
4. **TMB Substrate Solution:** One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
5. **Wash Concentrate 20X**: One (1) bottle containing 25 ml of concentrated buffer and surfactant.
6. **Dilution Buffer:** Two (2) bottles containing 30 ml of buffered protein solution.
7. **Stop Solution**: One (1) bottle containing 11 ml of 0.73 M phosphoric acid.

Materials required but not provided

1. Pipettes
2. Squeeze bottle for washing strips (narrow tip is recommended)
3. Reagent grade water and graduated cylinder
4. Tubes for sample dilution
5. Absorbent paper
6. ELISA plate reader with a 450 nm and a 620-650 nm filter (optional if results are read visually.

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 (four for calibrators plus number of samples) and place in strip holder.
2. Add 100 µl of each calibrator to wells 1-4, then and 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 ºC) for 10 minutes.
4. Shake out contents and wash 3 times with the diluted wash buffer.
5. Add 100 µl 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 100 µl of the Chromogen to every well. Mix by gently tapping strip holder.
9. Incubate at room temperature for 5 minutes.
10. Add 100 µl of the Stop Solution and mix by tapping strip holder.

RESULTS

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

Diagnostic Automation/Cortez Diagnostics, Inc.
23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302 USA Phone: 818.591.3030 Fax 818.591.8383
Email:onestep@rapidtest.com Website: www.rapidtest.com

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.

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 absorbence 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).

LIMITATIONS OF PROCEDURE

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.

PRECAUTIONS

1. 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.
2. 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.
3. 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.
4. Do not add azides to the samples or any of the reagents.

STORAGE

1. Reagents, strips and bottled components should be stored at 2-8 °C
2. Squeeze bottle containing diluted wash buffer may be stored at room temperature.

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ISO 13485
ISO 9001

Diagnostic Automation/Cortez Diagnostics, Inc.
23961 Craftsman Road, Suite E/F,
Calabasas, California 91302 USA

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