Tetanus
Cat # 8205-3

Intended Use
For the quantitative determination of serum antibody response to tetanus toxoid.

Summary
The causitive agent of tetanus is the organism *Clostridium tetani*. This organism and its spores can be found in a variety of sources such as soil and the intestinal contents of animals. The tetanus toxin, a product of the infection, is among the most potent poisons known to humans and can induce the disease in any age group of non, or poorly immunized individuals.

The determination of tetanus antitoxin in human serum samples has been conveniently carried out by the enzyme linked immunosorbent assay (ELISA). Unlike conventional methods, ELISA's offer increased sensitivity, rapid test results, reduction in costs, and elimination of animal inoculations necessary in other methods.

Principle of Procedure
The micro test wells are coated with tetanus toxoid antigen. 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) and a substrate (hydrogen peroxide) are 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

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Symbol</th>
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<tbody>
<tr>
<td>Test Strips</td>
<td>Microwells containing tetanus toxoid antigens - 96 test wells in a test strip holder.</td>
<td>MT PLATE</td>
</tr>
<tr>
<td>Enzyme Conjugate</td>
<td>One (1) bottle containing 11 ml of Protein A conjugated to peroxidase.</td>
<td>CONJ</td>
</tr>
<tr>
<td>Standards</td>
<td>Four (4) vials containing 2 ml of diluted positive human serum.</td>
<td>CAL</td>
</tr>
<tr>
<td>Chromogen</td>
<td>One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).</td>
<td>SUBS TMB</td>
</tr>
<tr>
<td>Wash Concentrate (20X)</td>
<td>One (1) bottle containing 25 ml of concentrated buffer and surfactant.</td>
<td>WASH BUF</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>Two (2) bottles containing 30 ml of buffered protein solution.</td>
<td>SPECM DIL</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>One (1) bottle containing 11 ml of 1 M phosphoric acid.</td>
<td>SOLN</td>
</tr>
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## Precautions

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.

## Storage Conditions

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

## 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.

## Collection and Preparation of Serum

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.
**Procedure**

**Materials Provided**
Tetanus Toxoid Serology Microwell ELISA Kit

**Materials Required But Not Provided**
- 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

**Performance of Test**
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 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.

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

**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.

**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).

References
   American Society for Microbiology. pp. 574-586.
2. Finegold, S.M., and W.L. George. “Miscellaneous Clostridial Infections”. Laboratory Diagnosis of
   of the Llama (Lama glama) to Tetanus Toxoid Vaccination”. Am J Vet Res. Aug 1989; Vol 50:
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   Antibodies in Human Serum to Escherchia coli, Tetanus Toxoid, and Diphtheria-Tetanus Toxoids:
   Normal Values for IgG, IgA, and IgM”. Diagnostic Immunology. 1986; vol. 4, pp. 17-23.
8. Craig, J. P. “Immune Response to Corynebacterium diphtheriae and Clostridium tetani”. Manual of

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<thead>
<tr>
<th>Date Adopted</th>
<th>Reference No.</th>
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<tr>
<td>2007-08-01</td>
<td>DA-Tetanus-2008</td>
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