ELISA kits available from ADI (see details at the web site)

930-100-TTH Human Anti-Tetanus Toxin/Toxoid IgG ELISA kit
930-120-TMA Mouse Anti-Tetanus Toxin/Toxoid IgA ELISA kit
930-130-TMG Mouse Anti-Tetanus Toxin/Toxoid IgG ELISA kit
930-140-TMM Mouse Anti-Tetanus Toxin/Toxoid IgM ELISA kit
930-210-TRG Rabbit Anti-Tetanus Toxin/Toxoid IgG ELISA kit
930-220-TRM Rabbit Anti-Tetanus Toxin/Toxoid IgM ELISA kit
930-310-TGG G. pig Anti-Tetanus Toxin/Toxoid IgG ELISA kit
930-320-TGM G. pig Anti-Tetanus Toxin/Toxoid IgM ELISA kit
930-410-TKG Monkey Anti-Tetanus Toxin/Toxoid IgG ELISA kit

Tetanus Toxoid/Toxin (TTX) ELISA for the measurement TTX in biological buffer
96 tests

Cat. #. VAC-TTX-310

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777
Email: service@4adi.com
Web Site: www.4adi.com
Tetanus Toxoid/Toxin (TTX) ELISA for the measurement TTX in biological buffer, 96 tests

<table>
<thead>
<tr>
<th>Kit Components, 96 tests</th>
<th>Cat #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Tetanus Toxoid (TTX) coated Strip plate (8x12 wells)</td>
<td>VACTTX-310P</td>
</tr>
<tr>
<td>Tetanus Toxoid (TTX) Std. A (0 mLf/ml), 0.65 ml</td>
<td>VACTTX-311A</td>
</tr>
<tr>
<td>Tetanus Toxoid (TTX) Std. B (15.6 mLf/ml), 0.65 ml</td>
<td>VACTTX-311B</td>
</tr>
<tr>
<td>Tetanus Toxoid (TTX) Std. C (31.2 mLf/ml), 0.65 ml</td>
<td>VACTTX-311C</td>
</tr>
<tr>
<td>Tetanus Toxoid (TTX) Std. D (62.5 mLf/ml), 0.65 ml</td>
<td>VACTTX-311D</td>
</tr>
<tr>
<td>Tetanus Toxoid (TTX) Std. E (125 mLf/ml), 0.65 ml</td>
<td>VACTTX-311E</td>
</tr>
<tr>
<td>Tetanus Toxoid (TTX) Std. F (250 mLf/ml), 0.65 ml</td>
<td>VACTTX-311F</td>
</tr>
<tr>
<td>Standards are provided in a stabilizing buffer containing 0.1% Proclin-300</td>
<td></td>
</tr>
<tr>
<td>Calibrated to NIBSC 02/232.</td>
<td></td>
</tr>
</tbody>
</table>

Sample/Conjugate Diluent, solution, 15 ml | VACTTX-312 |
Anti-TTX IgG-HRP Conjugate, 0.15 ml (100x), Dilute 1:100 with Conj. Diluent | VACTTX-313 |
Wash buffer (100X), 10 ml; dilute 1:100 with water | W B - 1 0 0 |
HRP substrate, Solution, 12 ml | 80091 |
Stop solution, 1 2 m l | 8 0 1 0 1 |
Instruction Manual #M-VAC-TTX-310 | 1

Tetanus, also called lockjaw, is a medical condition characterized by a prolonged contraction of skeletal muscle fibers. The primary symptoms are caused by tetanospasmin (also known as tetanus toxin); a neurotoxin produced by the Gram-positive, obligate anaerobic bacterium Clostridium tetani. Infection generally occurs through wound contamination and often involves a cut or deep puncture wound. As the infection progresses, muscle spasms develop in the jaw (thus the name "lockjaw") and elsewhere in the body. Infection can be prevented by proper immunization and by post-exposure prophylaxis. Nevertheless every year 400,000 - 800,000 persons die due to this infection. The majority of these persons live in under-developed countries. Tetanus begins when spores of Clostridium tetani enter damaged tissue. The spores transform into rod-shaped bacteria and produce the neurotoxin tetanospasmin. This toxin is inactive inside the bacteria, but when the bacteria die, it is released and activated by proteases. Active tetanospasmin is carried by retrograde axonal transport to the spinal cord and brain stem where it binds irreversibly to receptors at these sites. Ultimately, this produces the symptoms of the disease. Tetanus affects skeletal muscle, a type of striated muscle used in voluntary movement.

The ELISA for Tetanus Toxoid is the first commercial test to measure the active component of the vaccine (TTX) for formulations in vaccines. This kit has been validated with TTX used in Daptacel (DTAP, Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbe, Sanofi). Tetanus Toxoid is a component of several monovalent or multivalent vaccines: (Trihibit (DTAP/Hib), ActHib (Hib-PRP-T), Trihibit (DTAP/Hib), Daptacel (DTAP), Tripedia (DTAP), Td (Adult), DecavacTM (tetanus/Diphtheria), Adacel (tetanus, Diphtheria, Acellular Pertussis), DT (Pediatric) - Sanofi Pasteur; Pediarix (DTAP/HepB/IPV), Infanrix (DTAP), Boostrix (Tetanus, Diphtheria, Acellular Pertussis)-GlaxoSmithKline.

---

**CALCULATION OF RESULTS**

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on a graph paper by plotting net absorbance values of standards against appropriate TTX concentrations. Read off the TTX concentrations of the control and samples directly from the standard curve. If samples were diluted then the values should be multiplied by the dilution factor.

If ELISA reader software is being used, we recommend 4-parameter or 5-parameter curve.
WORKSHEET OF TYPICAL ASSAY

<table>
<thead>
<tr>
<th>Wells</th>
<th>Stds/samples</th>
<th>Mean $A_{450}$ nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A2</td>
<td>TTX Std. A (0 mLF/ml)</td>
<td>0.041</td>
</tr>
<tr>
<td>B1, B2</td>
<td>TTX Std. B (15.6 mLF/ml)</td>
<td>0.128</td>
</tr>
<tr>
<td>C1, C2</td>
<td>TTX Std. C (31.25 mLF/ml)</td>
<td>0.27</td>
</tr>
<tr>
<td>D1, D2</td>
<td>TTX Std. D (62.5 mLF/ml)</td>
<td>0.57</td>
</tr>
<tr>
<td>E1, E2</td>
<td>TTX Std. E (125 mLF/ml)</td>
<td>1.3</td>
</tr>
<tr>
<td>F1, F2</td>
<td>TTX Std. F (250 mLF/ml)</td>
<td>2.42</td>
</tr>
</tbody>
</table>

A typical std. assay curve (do not use this for calculating sample values). A complete standard curve must be run in every assay to determine sample values.

PRINCIPLE OF THE TEST

Tetanus Toxoid (TTX) ELISA kit is based on binding of TTX to an antibody coated on the plate and antibody-HRP conjugate. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of TTX present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microliter well ELISA reader at 450 nm. and the concentration of TTX in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader. Table top microfuge

PRECAUTIONS AND SAFETY INSTRUCTIONS

ADI TTX ELISA kit is intended for in vitro research use only. The reagents contain proclnin-300 (0.1%) as preservative; necessary care should be taken when disposing solutions. Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the website.

TMB (substrate), H2SO4 (stop solution), and Proclnin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SPECIMEN COLLECTION AND HANDLING

This kit is designed to measure the TTX in vaccine formulated in Alum (Aluminum Phosphate gel). Do not add azide or other preservatives to vaccines. This kit is not suitable to measure TTX adsorbed on alum). ADI has other kits to measure TTX in biological buffers.

REAGENTS PREPARATION FOR THE ASSAY

Dilute wash buffer (1:100) with distilled water (10 ml stock in 990 ml). Store at 4oC.

Dilute enzyme conjugate 1:100 (eg; 10 ul of HRP in 990 ul antibody conjugate diluent). For 96 samples, take 110 ul stock conjugate in 11 ml diluent). You need approx. 10 ml for 96 samples. Do not keep working stock of diluent beyond the assay. Prepare only in required amounts.

STORAGE AND STABILITY

The kit contents, if unopened, are stable at 2-8oC until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of shipping under appropriate storage conditions..
TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

All standards, controls, and samples should be tested in duplicate. Dilute wash buffer 1:100 with water. Dilute HRP conjugate 1:100 in 1X Conjugate buffer.

1. Dilute samples with sample diluent. Do not dilute standards. Pipet 100 ul stds and diluted samples into appropriate wells. Gently mix the plate for 5-seconds by tapping against the palm. Cover the plate and incubate for 60 minutes at room temperature.

2. Note: for ease of loading samples it is recommended that a second uncoated microwell plate should be used for sample dilution. This enables standards or samples to be transferred quickly to the ELISA plate using multichannel pipet.

3. Aspirate and wash the wells 4 times with wash buffer (300 ul/well/wash). We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Pipet 100 ul of diluted Ab-enzyme conjugate into each well. Mix gently for 5-10 seconds. Cover the plate and incubate for 30 minutes at room temperature.

5. Aspirate and wash the wells 4 times with wash buffer(same as in step 4).

6. Dispense 100 ul TMB substrate solution per well. Mix gently. Cover the plate and incubate on a plate shaker for 15 minutes at room temp. incubation time may be + 5 min so as to get maximum A450 =<2.00-3.00). Blue color develops in standards and positive wells.

7. Stop the reaction by adding 100 ul of stop solution to all wells at the same timed intervals as in step 8. Mix gently for 5-10 seconds to make ensure even color distribution. Blue color turns yellow.

8. Measure the absorbance at 450 nm using an ELISA reader. Color is stable for at least 1 hr after stopping.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

All vaccine samples must be tested at least 2 dilutions that are within the standards range. Samples containing TTX more than highest standards (250 mLF/ml) should be diluted further. The results obtained should be multiplied by the appropriate dilution factor.

QUALITY CONTROL

Standards and controls, if available, must perform as stated in the manual. This kit is tested, optimized, and calibrated with Daptacel/DTAP (Sanofi). This vaccine can be used as control.

PERFORMANCE CHARACTERISTICS

DETECTION LIMIT- Based on replicate determinations of the zero standard, the minimum TTX concentration detectable using this assay is ~7 mLF/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Specificity

The antibodies used in this kit are specific for C. Tetani Toxin or Toxoid with no reactivity with diphtheria or Pertussis or other toxoids. This kit is not suitable to measure TTX protein in alum formulations of the vaccines.