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

VAC-TTX-310 VacciGel Direct ELISA for the measurement of **Tetanus Toxoid** in Vaccines formulated in Alum, 96 tests

VAC-TTX-310 Tetanus Toxoid/Toxin (TTX) ELISA for the measurement **TTX** in biological buffer

VAC-DTX-200 VacciGel Direct ELISA for the measurement of **Diphtheria Toxoid** in Vaccines formulated in Alum, 96 tests

VAC-DTX-210 Diphtheria Toxoid/Toxin (DTX) ELISA for the measurement **DTX** in biological buffer

VAC-HBS-100 VacciGel Direct ELISA for the measurement of **Hepatitis B Vaccine** (HBsAg) formulated in Alum, 96 tests

VAC-HCG-500 VacciGel Direct ELISA for the measurement of **HCG** (contamination) in Vaccines formulated in Alum, 96 tests

VAC-PTX-400 VacciGel Direct ELISA for the measurement of **Pertussis Toxoid** in Vaccines formulated in Alum, 96 tests

VAC-PTX-410, Pertussis Toxoid/Toxin (PTX) ELISA for the measurement **PTX** in biological buffer

Instruction Manual No. M-VAC-TTX-310

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

Cat. #. VAC-TTX-310

For In Vitro Research Use Only

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

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

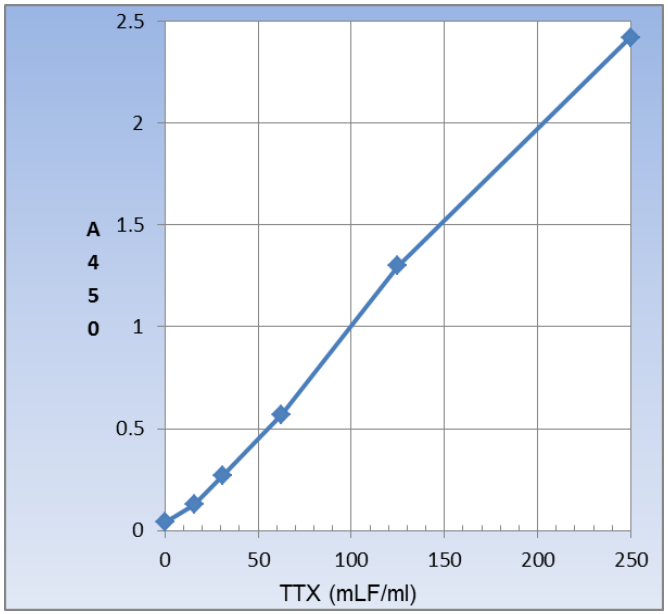
CALCULATION OF RESULTS

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-paramter or 5-parameter curve.

WORKSHEET OF TYPICAL ASSAY

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



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 microtiter 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 proclin-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 web site.

TMB (substrate), H2SO4 (stop solution), and Proclin-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 2-8^{O}C 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.