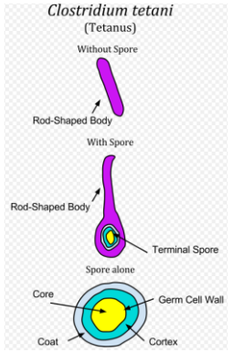
INTENDED USE

The Tetanus Toxin/Toxoid (TTX) ELISA Kit is an immunoassay for the quantification of Tetanus toxin or toxoid in cell culture, bioprocessing solutions, or in other appropriately qualified samples from tissue fluids (e.g., blood, saliva, mucosa). For research use only (RUO), not for diagnosis, cure or prevention of the disease.

GENERAL INFORMATION



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 that produces an anaerobic environment. As the infection progresses, muscle spasms develop in the jaw (thus the name "lockjaw") and elsewhere in the body.

Tetanus begins when bacterial spores enter damaged tissue. The spores transform into rod-shaped bacteria and produce the neurotoxin tetanospasmin. This toxin is inactive inside the bacteria, but is released and activated by proteases when the bacteria die. Active tetanospamin is carried by retrograde axonal transport to the spinal cord and brain stem where it binds irreversibly to receptors at these sites, and ultimately produces the symptoms of the disease.

Several **Tetanus vaccines** are available, as single antigen or as multivalent with antigens from other disease-causing microbes. Monitoring the efficacy of vaccines by determining the anti-Tetanus Ig levels in patients, including for clinical trials using new formulation of vaccines, is often required. The ADI Anti- Tetanus Toxoid ELISAs will quantify antibodies produced by vaccines, including Tetanus 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.

PRINCIPLE OF THE TEST

The Tetanus Toxin/Toxoid ELISA kit is based on the binding of tetanus toxin in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to HRP. After a washing step, substrate is added and color (blue) is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of tetanus toxin present in the sample. Stopping Solution is added to terminate the reaction, and A450nm is then measured using an ELISA microtiter well reader. The concentration of TTX in samples is calculated from a curve of TTX standards.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8^{o}C until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Tetanus Toxoid - HRP Conjugate Concentrate (100x) Part No. TTX-314, 0.15ml	in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8^{o}C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Anti-Tetanus Toxoid Microwell Strip Plate	TTX-311	8-well strips (12)	Coated with anti-tetanus toxoid, and post-coated with stabilizers.
Tetanus Toxin Standards			
10 mLf/ml 25 mLf/ml 50 mLf/ml 100 mLf/ml 200 mLf/ml	TTX-313B TTX-313C TTX-313D TTX-313E TTX-313F	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Five (5) vials, each containing purified tetanus toxoid; diluted in buffer with protein, detergents antimicrobial as stabilizers.
Positive Control [Tetanus Toxoid] range on label	TTX-312	0.65 ml	Tetanus toxoid of stated concentration range; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Antibody HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website: http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

ASSAY DESIGN AND SET-UP

Sample Collection and Handling
Culture medium, bioprocessing preparations, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference (See Limits of the Assay, page 6). For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature.

For all samples, clarify by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Assay Validation
Validate the performance of the sample antigen and matrix in the assay system for recovery and parallelism (see Limits of the Assay, page 6), as follows:

Recovery – a measure of the interference of the sample matrix (diluent effect) in providing accurate quantitation of the sample Tetanus Toxin relative to the Standard curve.

Prepare and run a series of dilutions of the sample antigen (concentrations that will fall within the Standard range) in Working Sample Diluent to determine the dilutions that give consistent and accurate quantitation. For most buffer solutions a minimum 5-fold sample dilution is usually sufficient.

Parallelism – dilutions of the sample should read equivalent values from the top and bottom of the Standard curve to provide good assay precision.

Prepare a dilution series of the sample antigen that gives complete recovery and falls within the full range of the Standard curve. Sample readings from the upper and lower regions of the curve should differ by less than 25%.

Plate Set-up
Bring all reagents to room temperature (18-30^{o} C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Tetanus Toxin HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8 ^{o} C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

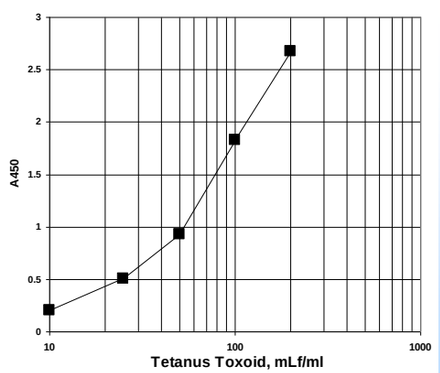
CALCULATION OF RESULTS

1. The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, Tetanus Toxin concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples.
3. On graph paper plot the mean OD of the standards (y-axis) against the concentration (mLf/ml) of Tetanus Toxin (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
4. The Tetanus Toxin concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
5. Multiply the values obtained for the samples by the dilution factor of each sample.
6. Samples producing signals higher than the 200 mLf/ml standard should be further diluted and re-assayed.

Typical Results:

Wells	Calibrators & Samples		**A450 nm**
A1, A2	Diluent Blank		0.04
B1, B2	10 mLf/ml	**Calibrator**	0.20
C1, C2	25 mLf/ml	**Calibrator**	0.51
D1, D2	50 mLf/ml	**Calibrator**	0.93
E1, E2	100 mLf/ml	**Calibrator**	1.83
F1, F2	200 mLf/ml	**Calibrator**	2.67
G1, G2	Positive Control [49 - 91]		1.29

Positive Control = **66.1** mLf/ml



PERFORMANCE CHARACTERISTICS

Specificity

The antibodies used in the kit are reactive with Tetanus toxin (tetanospasmin) and toxoid, and have neutralization activity in an *in* vivo assay. The assay is, therefore, specific for toxins and toxoids of Tetanus strains.

Standards

The tetanus toxoid used for standards is a WHO International Standard for the Tetanus Toxoid Flocculation Test, and is valued in Lf units, with 1 mLf unit = 1 ng/ml.

Precision

Samples containing low, medium and high concentrations of tetanus toxoid were assayed as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficients of variation were calculated for the concentrations using a point-to-point curve-fitting program.

Tetanus Toxin concentrations were measured with good between-assay (5.6 to9.9 %CV) reproducibility.

Sample	**Tetanus Toxoid** mLf/ml	**Inter-assay** %CV
High Concn	122	**5.6**
Medium Concn	67.5	**6.5**
Low Concn	29.0	**9.9**

LIMITS OF THE ASSAY

1. Tetanus toxoid is used as protein conjugate of several vaccines and would be detected in the assay. The dilution curve of the PRP-tetanus toxoid conjugate of the *Hemophilus influenzae* type b vaccine by Sanofi-Pasteur (ACTHIB) was measured as closely parallel to the Tetanus toxoid standard curve; the operator should determine parallelism and recovery for the specific tetanus toxoid preparation being investigated.

2. The Tetanus toxoid in the DPT vaccine is absorbed essentially entirely on the aluminum hydroxide adjuvant of each vaccine preparation. In this bound form, the Tetanus toxoid is not available for detection in this ELISA assay. The antibodies used in this assay, however, have been shown to quantify the vaccine antigen using another immunoassay format designed for use with bound Tetanus toxoid.

3. Toxins that are incomplete in sequence, or aggregated and/or associated with other biomolecules, or toxoids that are over treated with formaldehyde, may not produce dilution curves **parallel** with the Standard curve. For cases of non-parallelism, it may be useful to establish an alternative Standard curve using the altered Tetanus Toxin preparation.

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls A Positive Serum Control is provided with the kit, assigned with a tetanus toxoid concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Sample Diluent blank should also be run; OD should be <0.3 and lower than 10 mLf/ml Standard OD.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-uniform or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. Do not rely on results generated from an assay with these issues.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

Equipment Precision of results relies on uniform and effective washing techniques; an automatic washer may be used. ELISA reader and pipettes should be properly calibrated.

RELATED ITEMS

Catalog	Product Description
930-TTX-AG1	Tetanus Toxin/Toxoid ELISA Kit, quantitative
930-100-TTH	Human Anti- Tetanus Toxin IgG ELISA Kit
930-410-TKG	Monkey Anti- Tetanus Toxin IgG ELISA Kit
960-130-TMG	Mouse Anti- Tetanus Toxin IgG ELISA Kit
960-140-TMM	Mouse Anti- Tetanus Toxin IgM ELISA Kit
960-120-TMA	Mouse Anti- Tetanus Toxin IgA ELISA Kit
960-210-TGG	Rabbit Anti- Tetanus Toxin IgG ELISA Kit
VAC-TTX-300	VacciGel ™ | Direct ELISA for the detection and measurement of Tetanus Toxin adsorbed onto Alhydrogel

Instruction Manual No. M-930-TTX-AG1

Tetanus Toxin

ELISA Kit Cat. No. 930-TTX-AG1

For Quantitation of Tetanus Toxin/Toxoid in Solution or buffers

For research use only (RUO), not for diagnosis, cure or prevention of the disease.

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ELISA Kit Components	**Amount**	**Part**
Tetanus Toxoid Coated Microwell Strip Plate	8-well strips (12)	TTX-311
Tetanus Toxoid Positive Control	0.65 ml	TTX-312
Tetanus Toxoid Standard 10 mLf/ml	0.65 ml	TTX-313B
Tetanus Toxoid Standard 25 mLf/ml	0.65 ml	TTX-313C
Tetanus Toxoid Standard 50 mLf/ml	0.65 ml	TTX-313D
Tetanus Toxoid Standard 100 mLf/ml	0.65 ml	TTX-313E
Tetanus Toxoid Standard 200 mLf/ml	0.65 ml	TTX-313F
Anti-Tetanus Toxoid HRP (100X)	0.15 ml	TTX-314
Sample Diluent Concentrate (20x)	10 ml	SD20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	930-TTX-AG1