PRODUCT SPECIFICATIONS

Assay Specificity

Purified tetanus toxoid is used to coat the microwells; stabilizing postcoat contains casein; thus, no other antibody specificity is detectable in the assay. The anti-mouse IgA HRP conjugate specifically detects IgA, and will not react with IgG, IgM or IgE class antibodies.

Assay Sensitivity

The tetanus toxoid coating level and the anti-mouse IgA HRP concentration are optimized to differentiate anti-Tetanus Toxoid IgA from background (non-antibody) signal with mouse serum samples diluted 1:100.

Species Reactivity

This kit is specific for detecting anti-tetanus IgA in mouse. ADI has other kits that are designed to work in rabbit, guinea pig, monkey, and human.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Diluents and Conjugate contain non-azide antimicrobials. 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 antimicrobials, if not already on file, can be requested or obtained from the ADI website.

ELISA Kits available from ADI

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

Mouse, Rabbit, Human, Monkey, Guinea pig ELISA kits for Diphtheria, Pertussis, Hepatitis, H. influenza b antibody detection kits are also available.

Instruction Manual No. M-930-120-TMA

Mouse Anti-Tetanus Toxoid IgA

ELISA Kit Cat. No. 930-120-TMA

For Quantitative Determination of Anti-Tetanus Toxoid IgA in Serum

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INTENDED USE

The Mouse Anti-Tetanus Toxoid IgA ELISA Kit detects and quantifies tetanus toxoid-specific IgA in mouse serum or plasma of vaccinated or immunized animals. This immunoassay is suitable for:

- Determining immune status relative to non-immune controls;
- Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization and timing;
- Qualifying and/or standardizing vaccine batches and protocols.

INTRODUCTION

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 Mouse Tetanus Toxoid IgA ELISA kit is based on the binding of mouse anti-tetanus toxoid in samples to tetanus toxoid immobilized on the microwells, and anti-tetanus toxoid IgA antibody is detected by anti-mouse IgA-specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti-tetanus toxoid IgA present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The activity of mouse antibody in samples is determined relative to mouse anti-tetanus toxoid calibrators.

INTERPRETATION OF RESULTS (continued)

Method II. Titers from Sample Dilution Curves

The titer of antibody activity calculated from a dilution curve of each sample is recommended when sample curves are not parallel to each other and/or the Calibrator curve. Best precision can be obtained using the following guidelines:

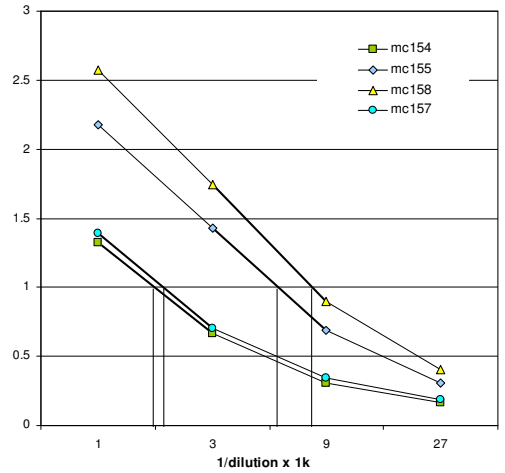
1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. A Calibrator value in the mid-OD range can be used to normalize inter-assay values.

Calculations

1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index
   = **Total IgA Antibody Activity Units**

Typical Results:

II. A **1.0 OD** Index was used to determine titer of 4 antibodies.



Titer Values

mc154 = 1.72 kU
mc155 = 5.70 kU
mc157 = 1.85 kU
mc158 = 7.90 kU

INTERPRETATION OF RESULTS

Quality Control & Expected Results

Sample Diluent Blank: < 0.3 OD; higher ODs suggest inadequate washing. If the OD of the Blank is greater than the lowest Calibrator, re-run the assay.

Non-immune/ Non-vaccinated Serum: Pre-immune/ non-immune sera at 1/100 dilutions should produce ODs lower than the low Calibrator. Very low antibody levels may be assayed at dilutions less than 1/100; because net ODs will be elevated, compare the results with pre-immune controls at the same dilutions.

Positive Control: Calculation: Positive Control, net OD ÷ 20 U/ml Calibrator, net OD x 20 U/ml = **Activity, U/ml**. Significant deviation from the U/ml range on the vial indicates poor assay precision; re-run the assay.

Calibrators: ODs should be in ascending order with low-to-high range > 1.0 OD. Variance of replicate values >15-20% indicates invalid precision for the run.

Quantitation

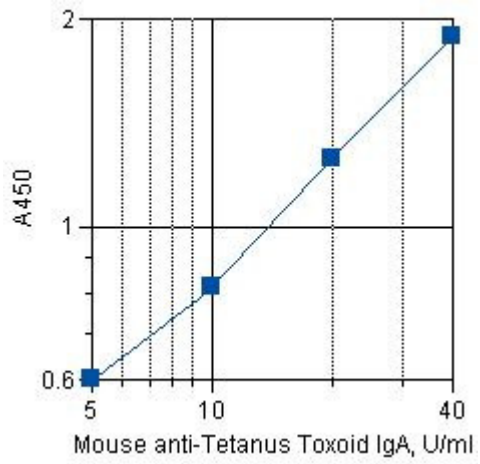
Several data reduction methods may be considered for presenting anti-tetanus toxoid results and for comparing experimental or treatment groups.

Method I. Use of a Calibrator Curve

When the dilution curves of samples are parallel to the Calibrator curve, the anti-tetanus toxoid activity units may be determined by interpolating from the curve and multiplying the value by the sample dilution (blank OD does not need to be subtracted).

Note: When sample dilution curves are **not parallel** with the Calibrator curve (which occurs frequently due to avidity variations), different values are obtained from different regions of the curve. This introduces a level of imprecision when comparing data from experimental and control groups and in replicate assays. For optimal reproducibility, use the titering **Method II**.

Typical Calibrator Curve



KIT CONTENTS

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

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
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-Mouse IgA - HRP Conjugate Concentrate (100x) Part No. MsrH-Ag, 0.15ml	Peroxidase conjugated anti-mouse IgA in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Antibody HRP Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amount	Contents
Tetanus Toxoid-coated Microwell Strip Plate	930-111	8-well strips (12)	Coated with tetanus toxoid, and post-coated with stabilizers.
Anti-Tetanus Toxoid IgA Calibrators			
5 U/ml 10 U/ml 20 U/ml 40 U/ml	930-123B 930-123C 930-123D 930-123E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing mouse anti-tetanus toxoid levels in arbitrary activity Units; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
Mouse x-Tetanus Toxoid IgA **Positive Control**	930-122	0.65 ml	Mouse anti-tetanus toxoid IgA, diluted in buffer with protein, detergents and antimicrobial as stabilizers. Value range on label calculated as shown on page 5.
Antibody HRP Diluent	TBT	12 ml	Buffer with protein, detergents and antimicrobial as stabilizers. Use as is for HRP dilution.
Sample Diluent	TBTm	60 ml	Buffer with protein, detergents and antimicrobial as stabilizers. Use as is for sample dilution.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% 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 Anti-Mouse IgA-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml 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.

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Serum, plasma and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately, store refrigerated with antimicrobial, or frozen for long-term storage.

Samples, Calibrators and Controls

Dilute **Samples** in Working Sample Diluent according to expected anti-tetanus toxoid activity levels; for serum: dilute at least 100-fold (e.g., 10ul sample + 990 ul Diluent) for reduced nonspecific signals. At least 2 dilutions of each sample is recommended in order to determine if reading values from the Calibrator curve is valid (see page 5).

Do not dilute the **Calibrators**. Include Sample Diluent as a Negative Control Blank to determine proper assay performance (signal should be < 0.3 OD) and to subtract from sample and standard values to obtain net OD. Internal **Controls** that represent the lab's expected results should also be included in each assay run.

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 8 Calibrator wells and 2 wells for each sample and control to be assayed, unless the titering method is used. Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.

Note:

- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes before sample addition. Aspirate or dump the liquid and pat dry on a paper towel.

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 standards, 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-Mouse IgA-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.