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

Catalog#	ProdDescription
930-100-TTH	Human Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests,
930-110-TTM	Mouse Anti-Tetanus Toxin/Toxoid Ig's (G+A+M) ELISA kit, 96 tests,
930-120-TMA	Mouse Anti-Tetanus Toxin/Toxoid IgA ELISA kit, 96 tests, Quantitative
930-130-TMG	Mouse Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
930-140-TMM	Mouse Anti-Tetanus Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
930-200-TTR	Rabbit Anti-Tetanus Toxin/Toxoid Ig's (G+A+M) ELISA kit, 96 tests,
930-210-TRG	Rabbit Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
930-220-TRM	Rabbit Anti-Tetanus Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
930-310-TGG	G. pig Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
930-320-TGM	G. pig Anti-Tetanus Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
930-410-01N	Monkey (cynomolgous) Anti-Tetanus Toxin/Toxoid IgG negative serum
930-410-02P	Monkey (cynomolgous) Anti-Tetanus Toxin/Toxoid IgG positive serum
930-410-TKG	Monkey Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests,
930-415-01N	Monkey (cynomolgous) Anti-Tetanus Toxin/Toxoid IgM negative serum
930-415-02P	Monkey (cynomolgous) Anti-Tetanus Toxin/Toxoid IgM positive serum
930-415-TKM	Monkey Anti-Tetanus Toxin/Toxoid IgM ELISA kit, 96 tests,
930-500-HTG	Horse Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
930-510-HFA	Horse Anti-Tetanus Toxin/Toxoid IgG-Fab2 ELISA kit, 96 tests,
930-TTX-AG1	Tetanus Toxoid/Toxin (TTX) ELISA for the measurement TTX in biological buffer, 96 tests

AV-9105-25	Tetanus Toxoid from C. tetani purified, vaccine grade
RP-343	Recombinant Anti-Tetanus Toxoid scFv IgG
SP-66125-5	Tetanus toxin (TT) peptide
SP-86741-1	TET 830 modified/T - helper epitope from tetanus toxoid
TSST11-A	caT# change to #TTOX12-A; Anti-C. tetani purified toxin IgG (tetanus shock toxin)

TTOX12-A	Anti-C. tetani purified toxin IgG (tetanus shock toxin)
TTOX13-A	Duplicate item same as TTOX11-A; Anti-C. tetani purified toxin IgG (tetanus shock toxin)

TTOX14-M	Monoclonal Anti-C. tetani purified toxin IgG (tetanus shock toxin)
TTOX15-N-25	Tetanus Toxoid from C. tetani purified
TTOX15-S	Anti-C. tetani purified toxin IgG (tetanus shock toxin)

TTOX18-A	Anti-C. tetani purified toxin/Toxoid IgG (Tetanus antitoxin, neutralizing, 300 IU/ml)

TTOX19-A	Anti-C. tetani purified toxin/Toxoid IgG (Tetanus antitoxin, neutralizing, 750 IU/ml)

TTOX20-Fab2	Anti-C. tetani purified toxin/Toxoid IgG (Fab2), Tetanus antitoxin (neutralizing)

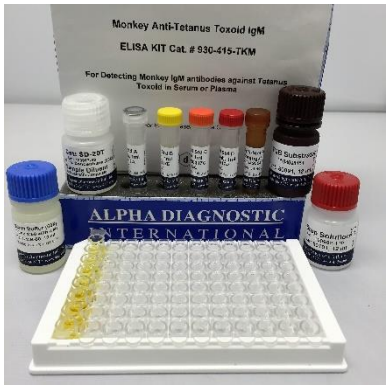
VAC-TTX-50	VacciGel Direct ELISA for the measurement of Tetanus Toxoid in Vaccines formulated in Alum, 50 tests

Instruction Manual No. M-930-415-TKM

Monkey Anti-Tetanus Toxoid IgM

ELISA KIT Cat. # 930-415-TKM

For Detecting Monkey IgM antibodies against Tetanus Toxoid in Serum or Plasma



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

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Monkey anti-Tetanus Toxoid (TTX) IgM ELISA KIT Cat. # 930-415-TKM (96 tests)

Kit Components (96 tests)	Cat #
Tetanus Toxoid (TTX) antigen coated strip plate, (8x12 strip or 96 wells) # 930-411	1 plate
Monkey Anti-TTX IgM Std. A IgM (0.08 u/ml) 1 mL) #930417A (clear cap)	1 vial
Monkey Anti-TTX IgM Std. B IgM (0.4 u/ml) 1 mL) #930417B (yellow cap)	1 vial
Monkey Anti-TTX IgM Std. C IgM (2.0 u/ml) 1 mL) #930417C (orange cap)	1 vial
Monkey Anti-TTX IgM Std. D IgM (10.0 u/ml) 1 mL) #930417D (red cap)	1 vial
Anti-Monkey IgM-HRP Conjugate (100X), (0.15 ml) #930418 brown vial	1 vial
Sample/HRP Conj. diluent (10 ml), #SD-20T (20X) clear cap bottle)	1
Wash buffer (50X) 15 ml # WB-50 (blue cap BOTTLE)	1
TMB Substrate Solution, 12 ml #80091 (brown bottle)	1
Stop Solution, 15 ml # 80101 (red cap)	1
Complete Instruction Manual # M-930-415-TKM	1

Intended Use

ADI Monkey Tetanus Toxoid IgM Antibody ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgM antibodies against Tetanus Toxoid in monkey serum or plasma. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

Introduction

Tetanus is a disease caused by the toxin from Clostridium tetani. Through better hygienic conditions and a wide prophylaxis by vaccination, the disease rate could be decreased worldwide. Nevertheless, every year 400,000 - 800,000 persons die by this infection. The majority of these persons live in under-developed countries. The protection through vaccination is very rare in older persons, because Tetanus antitoxin levels decline with age. The immunity against Tetanus has a vital significance for a lot of actions in business and free time. Sufficient protection is achieved by vaccination and following booster injections. Protection begins at a level of 0.1 IU/mL of anti-Tetanus Toxoid.

There is only a very low vaccination risk. Nevertheless, it is advisable to detect the immunity with a qualified test before boostering. By this way it is possible to prevent the patient of side effects like local swelling, pain and fever. Failure to respond to one or more antigens can sometimes be observed in patients with normal or high levels of all immunoglobulins, and in patients with isolated immunodeficiencies. Thus, normal immunoglobulin concentrations do not exclude antibody deficiency, and response to antigenic stimulation should be tested. If antibody determinations are performed over an extended period of time after priming and boostering, abnormalities in the rate of decline of cellular interactions as well as disorders in peak titers.

INTERPRETATION OF RESULTS

There are no guidelines for monkey samples. We suggest that the user make their own guidelines to determine the vaccine status of the animals or the exposure of monkey to tetanus.

EXPECTED MONKEY VALUES

N	A450 values at 1:100 dilutions	Results
18	0.05-0.200	<0.3 U/ml
3	0.3-0.1.1	>0.4-0.8 U/ml

21 samples (adult, mixed samples, vaccine status unknown) were tested in the ELISA. Most samples showed very low levels of tetanus IgM and 3 samples showed slightly elevated antibody levels.

Monkey Anti-Tetanus IgG and IgM –ve and +ve sera

ADI has screened large number of cynomolgous monkey sera samples to identify negative and positive containing IgM antibodies to tetanus toxoid. These can be purchased separately.

930-410-01N	Monkey (cynomolgous) Anti-Tetanus Toxin/Toxoid IgG negative serum
930-410-02P	Monkey (cynomolgous) Anti-Tetanus Toxin/Toxoid IgG positive serum
930-410-03N	Monkey (Rhesus) Anti-Tetanus Toxin/Toxoid IgG negative serum
930-410-04P	Monkey (Rhesus) Anti-Tetanus Toxin/Toxoid IgG positive serum
930-410-05N	Monkey (Baboon) Anti-Tetanus Toxin/Toxoid IgG negative serum
930-410-06P	Monkey (Baboon) Anti-Tetanus Toxin/Toxoid IgG positive serum
930-410-TKG	Monkey Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
930-415-01N	Monkey (cynomolgous) Anti-Tetanus Toxin/Toxoid IgM negative serum
930-415-02P	Monkey (cynomolgous) Anti-Tetanus Toxin/Toxoid IgM positive serum
930-415-03N	Monkey (Rhesus) Anti-Tetanus Toxin/Toxoid IgM negative serum
930-415-04P	Monkey (Rhesus) Anti-Tetanus Toxin/Toxoid IgM positive serum
930-415-05N	Monkey (Baboon) Anti-Tetanus Toxin/Toxoid IgM negative serum
930-415-06P	Monkey (Baboon) Anti-Tetanus Toxin/Toxoid IgM positive serum
930-415-TKM	Monkey Anti-Tetanus Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative

PERFORMANCE CHARACTERISTICS

Intra-Assay-Precision 6.9 % **Inter-Assay-Precision** 10.4 %
Inter-Lot-Precision 7.4-13.4% **Analytical Sensitivity** 0.004 U/mL
Linearity 77-114 %

Interferences

No interferences to bilirubin up to 0.3 mg/mL; Hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL.

Cross Reactivity

No cross reactivity to Corynebacterium diphtheria.

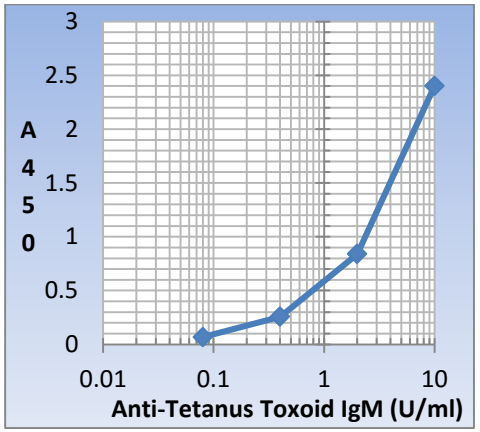
This kit is designed to detect monkey (cyno) IgM to tetanus toxoid. It doesn't detect IgM or other monkey antibody isotype. There is substantial reactivity between monkey (rhesus, cynomolgous) and baboons etc. This kit has been tested and reacted with anti-TTX in Rhesus, Cynomolgous, and Baboon serum sample. It also shows significant cross-reactivity with human IgM but not with other species such as mouse, rat, and g. pig. ADI has separate species specific kits for detecting anti-tetanus in various species (see page 7 or contact ADI if a kit is not listed.).

References: Chandler, H.M., et al (1984) A new rapid semi-quantitative enzyme immunoassayfor tetanus. 8;137; Eisel, U.. et al (1986) Tetanus Toxin primary structure 5; 2495.

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	U/mL	Mean A450	Net Mean A450
A1, A2	**Blank**	0.00	0.1	0.00
B1, B2	Std. A	0.08	0.079	0.068
C1, C2	Std. B	0.4	0.266	0.256
D1, D2	Std. C	2.00	0.94	0.84
E1, E2	Std. D	10.00	2.50	2.40

NOTE: These data are for **demonstration purpose only**. It must not be used to determine the sample results.



/Arif/3-ADI/930-415-TKM

CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log. For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The initial dilution of the samples has been taken into consideration when reading the results from the graph. Therefore, antibody concentration of the samples can be directly read using the standard curve.

Samples showing concentrations above the highest standard have to be re-tested at a dilution of 1:400 or higher. The result in IU/mL read from the calibration curve for this sample must then be multiplied by a factor of 4.

PRINCIPLE OF THE TEST

Alpha Diagnostic's Tetanus Toxoid IgM antibody test kit is based on the principle of the enzyme immunoassay (EIA). Tetanus antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgM antibodies of the serum and the immobilized Tetanus Toxoid antigen takes place. After 1 hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then antibody-peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is added for the development of a blue color in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting color is measured using ELISA reader at 450 nm. The concentration of the IgM antibodies is directly proportional to the intensity of the color.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. Bidistilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm).

PRECAUTIONS

Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken. Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly. All reagents have to be brought to room temperature (18 to 25 °C) before performing the test. Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided. It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions. When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time. In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used. No reagents from different kit lots have to be used, they should not be mixed among one another. All reagents have to be used within the expiry period. In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation. The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

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 Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results. For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent). We recommend preparing initial sample dilution of 1:10 first (10 ul sample and 90 ul sample dilutent). This can be stored at 4oC for weeks to allow full testing of the samples without freezing and thawing. Additional testing dilutions of 1:50 or 1:100 can be made from 1:10 stock (e.g. to make 1:100 test dilution, dilute 1:10 stock 10-fold or 25 ul of 1:10 and 230 ul of sample diluent to prepare 250 ul for testing in duplicate.

Note: if testing non-vaccinated samples, we recommend testing 1:100 diluted samples. Vaccinated monkeys can be initially tested at 1:100-1:1000 or more and then further sample dilutions are tested depending upon the antibody level.

REAGENTS PREPARATION

1. **Dilute Wash buffer** 1:50 with water. Store diluted buffer at 4oC for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37oC for 15 minutes or in warm water.

2. **Dilute 100X antibody-HRP Conjugate** with conjugate diluent (prepare 1 ml for 1 strip or 10 ml for full plate; 10 ul of 100X conjugate in 990ul of 1X sample/Conj. diluent or 100 ul in 9.90 ml of diluent). Prepare the conjugate as needed and do not store 1X diluted conjugate beyond the test date.

3. **Dilute 20X Sample/Conjugate Diluent** with distilled water (1 ml stock in 19 ml water). Store 1X diluent at 4oC until used. This is used for diluting samples and the 100X conjugate.

All reagents must be at room temperature prior to their use.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8^{o}C or stored frozen in small aliquots and should be stable for 3 months.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. **All samples should be diluted 1:101 (5 ul samples in 500 ul sample diluent)**. It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate.

Label or mark the microtiter well strips to be used on the plate. Prepare 1X working solution of wash buffer, sample and conjugate diluent, and HRP conjugate (see page 3).

1. Dispense 100 ul diluent in 1 well to be used as blank. Pipet **100 ul of** Prediluted standards and samples (diluted 1:100 or more) into appropriate wells in *duplicate*. Cover the plate, mix gently for 5-seconds and incubate at room temp for 60 min.

2. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 250-300 ul of 1X wash buffer. 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.

3. Add **100 ul 1x anti-IgM-HRP conjugate** to all wells leaving one empty for the substrate blank. Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temp (25-28oC).

4. Wash the wells 3 times as in step 3.

5. Add **100 ul TMB substrate solutions**. Mix gently for 5-10 seconds. Cover the plate and incubate for **15 minutes** at room temp. Blue color develops in positive controls and samples. **Note**: It is possible to change the incubation time $\pm$ 5 mins so as to get the maximum color after stopping the reaction to 2.00-3.00 as many readers do not read linear above 2.00.

6. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 5-10 seconds to have uniform color distribution (blue color turns yellow).

7. Measure the **absorbance at 450 nm** (630 nm reference) using an ELISA reader within 30 min.

Quality Control

The test results are only valid if the test has been performed following the instructions. All standards must be found within the acceptable ranges. Blanks must not exceed >0.300 and the high std must be >1.00. Repeat the test for significant deviations and report to ADI.

ADI has separate monkey anti-tetanus IgG/IgM negative and positive sera that can be purchased for additional testing.