Instruction Manual No. M-930-100-TTG+

Human Anti-Tetanus Toxoid IgG

ELISA KIT Cat. # 930-100-TTG+, 96 Tests

For Detecting Human IgG antibodies against Tetanus Toxoid in Serum or Plasma

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

India Contact:

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034
Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@atzlabs.com
Web: www.atzlabs.com

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Tetanus Toxiod IgG Plus ELISA KIT Cat. # 930-100-TTG +(96 tests)

Kit Components (96 tests)	Cat #
Tetanus Toxoid antigen coated strip plate, (8x12 strip or 96 wells) # 930-101	1 plate
Standard A (0 IU/ml; 2mL) #930-102A	1 vial
Standard B (0.1 IU/ml 2mL) #930-102B	1 vial
Standard C (0.5 IU/ml 2mL) #930-102C	1 vial
Standard D (1.0 IU/ml 2mL) #930-102D	1 vial
Standard E (5.0 IU/ml 2mL) #930-102E	1 vial
Tetanus Toxoid Control, # 930-100CT, 2 mL	1 vial
Anti-Human IgG-HRP Conjugate, (20 ml) #930-107	1 bottle
Sample Diluent, 100 ml #930-100SD	1 bottle
Wash buffer (20X), 50 ml # 930-100WB	1 bottle
TMB Substrate Solution, 15 ml #930-100SS	1 bottle
Stop Solution, 15 ml # 930-100ST	1 bottle
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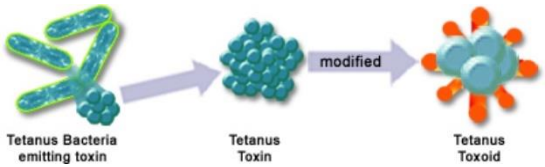
Intended Use

ADI Human Tetanus Toxoid IgG Antibody ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgG antibodies against Tetanus Toxoid in serum and plasma (citrate, heparin). 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

Quality Control

The test results are only valid if the test has been performed following the instructions. All standards and kit controls must be found within the acceptable ranges as stated on the vials. The positive control must show at least double the OD of the cut-off standard. If criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. In case of any deviation the following technical issues should be proven (reagents, protocol, equipments, etc).

PERFORMANCE CHARACTERISTICS

Intra-Assay-Precision <6.9%

Inter-Assay-Precision <10.49%

Analytical Sensitivity 0.02 IU/mL

Inter-Lot-Precision: 7.4-13.4%

Clinical Sensitivity: 90 %

Recovery: 76-107 %

Linearity: 77-114 %

Measurement range 0.02 IU/mL – 5.0 IU/mL.

Interferences:

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin

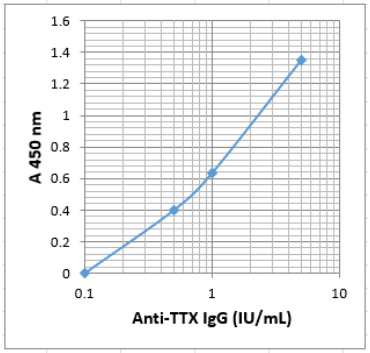
References

Ambrosch, F et al (1984) Micro-ELISA Methode zur Bestimmung der Tetanus-Antikorper, A258; Chandler, H.M., et al (1984) A new rapid semi-quantitative enzyme immunoassayfor tetanus. 8;137; Ehrengut W., et al (1970) Reaktionen der Wundstarrkrampfimpfung: 95; Eisel, U.. et al (1986) Tetanus Toxin primary structure 5; 2495.

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	IU/mL	Mean A450
A1, A2	**Blank (Standard A)**	0	0.064
B1, B2	Standard B	0.1	0.151
C1, C2	Standard C	0.5	0.400
D1, D2	Standard D	1.0	0.610
E1, E2	Standard E	5.0	1.402

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



CALCULATION OF RESULTS

The ready-to-use standards of the Tetanus Toxoid antibody kit are defined and expressed in International Units (IU/mL) and are calibrated in accordance with the Who International Standard; "1st International Standard for Tetanus Immunoglobulin, Human"; NIBSC Code: TE-3. Consequently, for a given subject follow-up controls become possible. For this evaluation the absorptions of the standards and controls are graphically drawn point-to-point against their concentrations. From the resulting reference curve, the concentration values for each sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs. As curve fit point-to-point has to be chosen.

Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

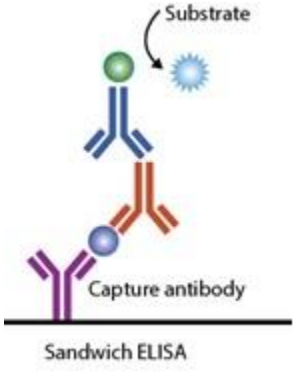
- **Substrate-Blank:** Absorbance value **< 0.100**
- **Standard A**: Absorbance value **< 0.200**
- **Standard B:** Absorbance value **> 0.050**
- **Standard C:** Absorbance value > **Standard B**
- **Standard D:** Absorbance value > **Standard C**
- **Standard E:** Absorbance value **> 0.800**
- **Control: Range mentioned on vial IU/ml**

Standard A < Standard B < Standard C < Standard D < Standard E

If these criteria are not met, the test is not valid and must be repeated.

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.

PRINCIPLE OF THE TEST



Alpha Diagnostic's Tetanus Toxoid IgG 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 IgG antibodies of the serum and the immobilized Tetanus Toxoid antigen takes place. After a one-hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove inbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 15 minutes, inducing the development of a blue dye 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 dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG 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

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. 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).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (Citrate, 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. Heat inactivation of samples is not recommended.

For the performance of the test the samples (not the standards) have to be diluted 1:100 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent). Do not dilute the calibrators.

REAGENTS PREPARATION

1. **Dilute Wash buffer (20X)** 1:19 with distilled water. (**e. g. 10 mL Washing Buffer + 190 mL distilled water.**) Store diluted buffer at 4ºC for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 degrees C for 15 minutes.

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 12 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 1:100 (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. **Standards are ready-to-use.**

1. Label or mark the microtiter well strips to be used on the plate. Dilute the wash buffer with water (1:20),

2. Pipet **100 ul of ready-to-use** standards, controls, and samples (diluted 1:100) into appropriate wells in *duplicate*. Leave 1 well to be used as blank.. See worksheet of a typical set-up on page 5. Cover the plate, mix gently for 5-seconds and incubate at 37°C for **60 min.**

3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 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.

4. Add **100 ul anti-IgG-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 (**20-25°C**).

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

6. Add **100 ul TMB substrate solution**. Mix gently for 5-10 seconds. Cover the plate and incubate for **15 minutes** at room temp. (20-25 °C) in the dark. Blue color develops in positive controls and samples.

7. 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).

8. Measure the **absorbance at 450 nm** using an ELISA reader within 60 min.

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. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate.

The unused strips should be stored in a sealed bag at 4^{O}C. Do not touch the bottom of the wells.