



## Instruction Manual

# Tetanus Toxoid IgG ELISA

**Enzyme immunoassay** based on microtiter plate  
for the detection and quantitative determination  
of human IgG antibodies against Tetanus Toxoid  
in serum and plasma



Cat. No.: ILE-TET01  
Storage: 2-8°C  
For in-vitro diagnostic use only

September 2010

<b>Contents</b>	<b>Page</b>
1. Intended Use	3
2. General Information	3
3. Principle of the Test	3
4. Limitations, Precautions and General Comments	4
5. Reagents Provided	4
6. Materials Required but not Provided	5
7. Specimen Collection and Handling	5
8. Assay Procedure	6
9. Evaluation	7
10. Assay Performance	7
11. References	8

## Symbole und Übersetzungen / Symbols and Translations

<b>Symbol</b>	English	French	German	Italian	Spanish	Greek
<b>CAL</b>	Calibrator	Etalon	Kalibrator	Calibratore	Calibrador	Πρότυπο Διάλυμα
<b>CONJ</b>	Conjugate	Conjugué	Konjugat	Coniugato	Conjugado	Διάλυμα Συμπλόκου
<b>CONC</b>	Concentrate (<n>-fold)	Concentré (<n> fois)	Konzentrat (<n>-fach)	Concentrato (<n>-volte)	Concentrado (<n>-veces)	Συμπύκνωση (<n> φορές)
<b>SAMP DIL</b>	Sample Diluent	Diluant échantillon	Proben- verdünner	Diluyente del campione	Diluyente de muestra	Διάλυμα Αραίωσης Δειγμάτων
<b>STOP</b>	Stop Solution	Solution d'arrêt	Stopp-Lösung	Soluzione d'arresto	Solución de parada	Διάλυμα Αναστολής
<b>SUBS</b>	Substrate	Substrat	Substrat	Substrato	Sustrato	Διάλυμα Υποστρώ- ματος
<b>MT PLATE</b>	Microtiter plate	Microplaque	Mikrotiterplatte	Piastre	Placa microtiter	Μικρόπλακα
<b>WASH BUF</b>	Wash buffer	Tampon de lavage	Waschpuffer	Soluzione di lavaggio	Tampón de lavado	Πλυστικό Διάλυμα

## **1. Intended Use**

The IMMUNOLAB 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. Further applications in other body fluids are possible and can be requested from the Technical Service of IMMUNOLAB.

This assay is intended for in-vitro diagnostic use only.

Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

## **2. General Information**

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 boosting. 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 boosting, abnormalities in the rate of decline of cellular interactions as well as disorders in peak titers.

## **3. Principle of the Test**

The IMMUNOLAB 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 unbound 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 20 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.

#### 4. Limitations, Precautions and General Comments

- 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, and they should not be mixed with 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.

#### 5. Reagents Provided

Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

Components	Volume / Qty.
Tetanus Toxoid antigen coated microtiter strips	12
Standards with 0, 0.1, 1, 2.5 and 5 IU/mL	5 x 2 mL
Enzyme Conjugate	15 mL
Substrate	15 mL
Stop Solution	15 mL
Sample Diluent	60 mL
Washing Buffer (10×)	60 mL
Plastic foils	2
Plastic bag	1
Instruction Manual	1

### **5.1. Microtiter Strips**

12 strips with 8 breakable wells each, coated with a Tetanus Toxoid antigen. Ready-to-use.

### **5.2. Standards**

5 x 2 mL, human serum diluted with PBS, with 0, 0.1, 1, 2.5 and 5 IU/mL of IgG antibodies against tetanus. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

### **5.3. Enzyme Conjugate**

15 mL, anti-human-IgG-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane and 5 mg/L Proclin<sup>TM</sup>. Ready-to-use.

### **5.4. Substrate**

15 mL, TMB (tetramethylbenzidine). Ready-to-use.

### **5.5. Stop Solution**

15 mL, 0.5 M sulfuric acid. Ready-to-use.

### **5.6. Sample Diluent**

60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

### **5.7. Washing Buffer**

60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

### **5.8. Plastic Foils**

2 pieces to cover the microtiter strips during the incubation.

### **5.9. Plastic Bag**

Resealable, for the dry storage of non-used strips.

## **6. Materials Required but not Provided**

- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter Plate Reader (450 nm)
- Microtiter Plate Washer
- Reagent tubes for the serum dilution
- Bidistilled water

## **7. 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 (4-8°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).

## 8. Assay Procedure

### 8.1. Preparation of Reagents

**Washing Solution:** dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- Standards and samples should be assayed in duplicates.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 4-8°C.

### 8.2. Assay Steps

1. Prepare a sufficient amount of microtiter wells for the standards, controls and samples in duplicate as well as for a substrate blank.
2. Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** standards and controls respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.
4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
5. Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.
7. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
8. Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
9. Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).
10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

## 9. Evaluation

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

### Example

	OD Value	corrected OD	Mean OD Value
Substrate Blank	0.020		
Standard 1 (0 IU/mL)	0.043 / 0.041	0.023 / 0.021	0.022
Standard 2 (0.1 IU/mL)	0.128 / 0.124	0.108 / 0.104	0.104
Standard 3 (1 IU/mL)	0.783 / 0.789	0.763 / 0.769	0.766
Standard 4 (2.5 IU/mL)	1.635 / 1.611	1.615 / 1.591	1.603
Standard 5 (5 IU/mL)	2.113 / 2.129	2.093 / 2.109	2.102

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently **no reference values** which have to be found in other laboratories in the same way.

### 9.1. Quantitative Evaluation

The ready-to-use standards of the Tetanus Toxoid antibody kit are defined and expressed in International Units (IU/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for the standards in International Units are printed on the labels of the vials.

For a quantitative evaluation the absorptions of the standards are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs.

The dilution factor of the samples (1:101) has already been reconsidered in the concentration given for the standards.

### 9.2. Interpretation

The results of each patient sample can be assessed as follows:

< 0.1 IU/mL	basic immunisation recommended
0.1 – 1.0 IU/mL	to be controlled after 1-2 years
1.0 – 5.0 IU/mL	to be controlled after 2-4 years
> 5.0 IU/mL	to be controlled after 4-8 years

## 10. Assay Characteristics

Tetanus Toxoid ELISA	IgG
Intra-Assay-Precision	6.9 %
Inter-Assay-Precision	10.4 %
Inter-Lot-Precision	7.4 – 13.4 %
Analytical Sensitivity	0.004 U/mL
Recovery	76 – 107 %
Linearity	77 – 114 %
Cross-Reactivity	No cross-reactivity to <i>Corynebacterium diphtheriae</i>
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
Clinical Specificity	90 %
Clinical Sensitivity	90 %

## 11. References

1. Ambrosch, F. et al. Eine neue Mikro-ELISA-Methode zur Bestimmung der Tetanus-Antikörper. Zbl. Bakteriol., **A258**: 173 (1984).
2. Chandler, H.M. et al. A new rapid semi-quantitative enzyme immunoassay for tetanus. J. Infect., **8**: 137 (1984).
3. Ehrengut, W. Reaktionen der Wundstarrkrampfpfimpfung; Dtsch. Med. Wschr., **95**: 1799 (1970).
4. Eisel, U. et al. Tetanus toxin: primary structure, EMBO J., **5**: 2495 (1986).
5. Frühwein, N. et al. Bestimmung von Tetanus-Antikörpern im menschlichen Serum mit dem ELISA Test. Ärztl. Labor, **26**: 271 (1980).
6. Korger, G. et al. Tetanusimpfung - Verträglichkeit und Vermeidung von Nebenreaktionen. Klin. Wschr., **64**: 767 (1986).
7. Melville-Smith, M.E. et al. A comparison of ELISA with the toxin neutralisation test for the estimation of tetanus antitoxin. J. Biol. Standard., **11**: 137 (1983).
8. Müller, H.E. et al. Tetanus-Schutzimpfung - Indikation und Kontraindikation. Dtsch. Med. Wschr., **113**: 1326 (1988).
9. Schröder, J.P. et al. Serologische Bestimmung von Tetanus-Antitoxin mit einem Enzymimmunoassay. Wehrmed. Mschr., **34**: 222 (1990).
10. Schröder, J.P. et al. Tetanusimpfenschutz und Vermeidung von Nebenreaktionen bei Auffrischimpfungen. Dtsch. Med. Wschr., **117**: 1903 (1992).
11. Sedgwick, A.K. et al. Rapid quantitative microenzyme-linked immunosorbent assay for tetanus antibodies. J. Clin. Microbiol., **18**: 104 (1983).
12. Zastrow, K.-D. et al. Tetanus - Erkrankungen, Impfungen und Impfschäden in der BRD. Dtsch. Med. Wschr., **118**: 1617 (1993).