

# Tetanus ELISA

Enzyme immunoassay for the quantitative determination of Tetanus Ab  
in human serum and plasma.

REF

**RE57441**



**96**



**2-8 °C**

EU:

IVD



U.S.: *For research use only.  
Not for use in diagnostic procedures.*



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## 1. INTENDED USE

The IBL Tetanus Enzyme immunoassay is for the quantitative determination of Anti-Tetanus Toxoid Antibodies in Human Serum, Citrate-, EDTA- or Heparin- Plasma. When the humoral immune status has to be checked, if test certificates are not available or where incorrect vaccination intervals have been used and a decision has to be made whether a basic immunization or a booster have to be given or no vaccination is necessary. The ELISA is used when immune response has to be checked in case of certain underlying diseases (e. g. malignoma, AIDS, hematological diseases). Furthermore the ELISA is used when immune response has to be checked because the patient is undergoing treatment for certain underlying diseases (immune suppression, cytostatics, corticosteroids, X-ray) and to find the cause for certain vaccination reactions.

## 2. SUMMARY AND EXPLANATION

The number of tetanus infections in Europe and the U.S. has considerably decreased as a result of improved immunization rates. However, the outcome of this avoidable infectious disease is so dangerous (lethality 50%) that an individual protection by antibodies is required in order to guarantee immunity. Immunity through vaccination can only be guaranteed if clearly confirmed by a valid certificate of vaccination or if serological tests verify the presence of protecting antibodies. The ELISA technique is a sensitive indicator as to whether a basic immunization or boosters are needed. ELISA also identifies intervals between vaccinations and offers an opportunity to avoid negative reactions. The correct interval between vaccinations is not always observed and in casualty cases certificates of vaccination are not available. It is, therefore usual to assume most of the patients with injuries as unprotected. In this situation unnecessary boosters, frequently followed by hyper allergenic reactions, are often given. Serological determination of tetanus toxoid antibodies will indicate whether a basic immunization or a booster is necessary ("vaccination management"). This enables the physician to match immune status and active immunization for each patient individually. This reduces the frequency of side effects leading to an improved acceptance of boosters.

## 3. TEST PRINCIPLE

The IBL Tetanus ELISA is a two-step ELISA. Wells in the ELISA test strips are coated with tetanus toxoid. Diluted serum or plasma samples are incubated in the wells of the test strips (sample incubation). During the incubation period specific antibodies against tetanus toxoid are bound to the solid phase. Non-specific components are washed away. Conjugate reaction takes place during the second incubation phase (conjugate incubation). The tetanus toxoid peroxidase conjugate acts as a marker for the bound anti-tetanus toxoid antibodies. Unbound conjugate is removed by a second washing step. In the third incubation phase the substrate reaction takes place. The peroxidase from the conjugate transforms the substrate Tetramethylbenzidin (TMB) into a blue colored substance. To stop the reaction sulfuric acid is added and the colour changes to yellow. The colour intensity is directly proportional to the anti-tetanus toxoid antibody concentration. The optical density is measured at a wavelength of 450 nm using an ELISA reader. Using the standard curve or the IBL One Point Calibration anti-tetanus toxoid antibodies concentration can be calculated quantitatively.

## 4. WARNINGS AND PRECAUTIONS

1. For in-vitro diagnostic use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.

9. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

## 5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The unopened reagents are stable until the expiry date indicated. The microtiter strips are stable until the expiry date, if they are stored at 2-8°C in the tightly closed bag. The storage and stability of prepared reagents is stated in the corresponding chapters.

## 6. SPECIMEN COLLECTION AND STORAGE

### Serum, Plasma

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	≤ -20°C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.
Stability:	5 d	12 mon	

## 7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	<b>MTP</b>	<b>Microtiter Plate</b> Ready to use. Break apart strips. Coated with tetanus toxoid.
2 x 75 mL	<b>DILBUF</b>	<b>Diluent Buffer</b> Ready to use. Red colored. Contains: detergents, 0.005 % (w/v) thimerosal, 0.01 M Tris/HCl; pH 7.4.
1 x 0.6 mL	<b>ENZCONJ CONC</b>	<b>Enzyme Conjugate Concentrate (100x)</b> Blue colored. tetanus toxoid conjugated to peroxidase.
5 x 0.35 mL	<b>CAL 1-5</b>	<b>CAL 1-5 Concentrate</b> Contains: human serum, stabilizers, preservatives. <b>Concentrations are lot-specific as indicated on the bottle labels.</b> Calibrated against WHO std. TE-3.
2 x 0.35 mL	<b>Control LL</b> <b>Control HL</b>	<b>Control LL+HL Concentrate</b> Positive Control Serum, LL, "Low Level", HL, "High Level". Contains: human serum, stabilizers, preservatives. <b>Concentrations are lot-specific as indicated on the bottle labels.</b>
1 x 100 mL	<b>WASHBUF</b> <b>CONC</b>	<b>Wash Buffer, Concentrate (10x)</b> Contains: 0.1 M Tris/HCl pH 7.4; detergents, 0.01 % (w/v) thimerosal.
2 x 12 mL	<b>TMB SUBS</b>	<b>TMB Substrate Solution</b> Ready to use. Contains: TMB (tetramethylbenzidine).
1 x 12 mL	<b>TMB STOP</b>	<b>TMB Stop Solution</b> Ready to use. Contains: 0.5 M H <sub>2</sub> SO <sub>4</sub> .
2 x	<b>FOIL</b>	<b>Adhesive Foil</b>

Note: Wash Buffer, Diluent Buffer, substrate solution, stop solution can be exchanged with following products: FSME IgG (RE57401), FSME IgM (RE57411), Diphtherie (RE57431).

## 8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volume: 5, 20, 100, 500, 1000 µL
2. Vortex mixer
3. Tubes for sample dilution
4. Orbital shaker (200-900 rpm) (e.g. EAS 2/4, SLT)
5. 8-Channel Micropipettor with reagent reservoirs
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
8. Bidistilled or deionised water
9. Paper towels, pipette tips and timer
10. Software „One Point Calibration“ for evaluation using Microsoft Excel 5.0 or higher (software will be provided for free on request).

## 9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

## 10. PRE-TEST SETUP INSTRUCTIONS

### 10.1. Preparation of concentrated Components (Example for 32 wells)

Dilute/ dissolve	Component	with	Diluent	Relation	Remarks	Storage	Stability
80 µL	<b>ENZCONJ</b> <b>CONC</b>	8 mL	<b>DILBUF</b>	1:101	Mix carefully. (Prepare immediately before needed.)	18-25°C	1 h
10 mL	<b>WASHBUF</b> <b>CONC</b>	90 mL	<b>bidist. water</b>	1:10	Mix carefully.	2-8°C	2 - 3 months

**10.2. Dilution of Standards, Controls and Samples**

	to be diluted	with	Relation	Remarks
<b>CAL 1-5</b> <b>Control LL</b> <b>Control HL</b>	generally	<b>DILBUF</b>	1:101	e.g. 10 µL CAL/Control + 1000 µL
<b>Serum/Plasma</b>	generally	<b>DILBUF</b>	1:101	e.g. 10 µL Sample + 1000 µL

For the determination by **standard curve** calibrators 1-5 and control sera are needed. For determination by **one point calibration** instead of calibrators 1 - 5 use only calibrator 4 and control sera.

**11. TEST PROCEDURE**

1.	Pipette <b>200 µL</b> of diluted <b>Calibrator, Control and sample</b> into the respective wells of the Microtiter Plate (Calibrators should be placed in strips 1 and 2).
2.	Cover plate with adhesive foil. <b>Incubate 60 min at RT (18-25°C).</b>
3.	Remove adhesive foil. Discard incubation solution. Wash plate <b>3 x with 250 µL</b> of diluted <b>Wash Buffer</b> . Remove excess solution by tapping the inverted plate on a paper towel.
4.	Pipette <b>200 µL</b> of diluted <b>Enzyme Conjugate</b> into each well.
5.	Cover plate with adhesive foil. <b>Incubate 60 min at RT (18-25°C).</b>
6.	Remove adhesive foil. Discard incubation solution. Wash plate <b>3 x with 250 µL</b> of diluted <b>Wash Buffer</b> . Remove excess solution by tapping the inverted plate on a paper towel.
7.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
8.	Pipette <b>200 µL</b> of <b>TMB Substrate Solution</b> into each well.
9.	<b>Incubate 30 min at RT (18-25°C).</b>
10.	Stop the substrate reaction by adding <b>50 µL</b> of <b>TMB Stop Solution</b> into each well. Briefly mix contents by gently shaking the plate.
11.	<b>Measure</b> optical density with a photometer at <b>450 nm</b> (Reference-wavelength: 650 nm) within <b>10 min</b> after pipetting of the Stop Solution.

**12. QUALITY CONTROL**

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All kit controls must be found within the acceptable ranges as stated on the vial labels. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

**13. CALCULATION OF RESULTS**

Determination of anti-tetanus toxoid antibodies can be done either by standard curve or by one point calibration.

### 13.1. Determination of standard curve

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 Logisitics 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 concentration of the samples can be read from the standard curve.

The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.

Samples showing concentrations above the highest standard can be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

### 13.2. Determination by one-point calibration

The software „IBL One Point Calibration“ for evaluation using Microsoft Excel 5.0 or higher will be provided on request. After opening the Excel-file „IBL One Point Calibration“ enter

- the absorbance nominal value of calibrator 4.
- the measured absorbance of calibrator 4 (mean value).
- the absorbance range of calibrator 4.
- the reference curve coefficients A, B, C and D.

in the prepared cell. The absorbance of calibrator 4 must be in the indicated range (see QC certificate). Insert identification and absorbance mean values of the control sera and samples into the prepared calculation sheet. The correction of the absorbance and the calculation of the concentrations will be done automatically.

#### Validation criteria

The assay is valid if the absorbance of calibrator 4 is in the indicated range and if concentrations of the control sera are read within the stated range.

### 13.3. Checking of the Calculated Sample Concentrations

Samples with an absorbance exceeding that of the standard curve should be prediluted (1:5) with incubation buffer. The concentrations thus obtained have to be multiplied by the factor 5. The conversion of citrate plasma to serum values is achieved by multiplying the recorded concentrations with the factor 1.1.

## 14. INTERPRETATION OF RESULTS

In the literature [1, 3, 5, 6, 7] the following values for tetanus immunization are given: "no protection" at tetanus antibody concentrations below 0.01 IU/ml, "uncertain protection" at 0.01 to 0.1 IU/ml and "secure individual protection" at concentrations over 0.1 IU/ml. As tetanus antibody concentrations will decrease over time, according to literature [7]. The following recommendations for test evaluation are given in the table below. The test result has to be judged by the physician in any single case taking into account the specific test precision. If vaccination is controlled by a serological test it is recommended to certify the tetanus antibody concentration in the certificate of vaccination.

IU/mL	Interpretation
< 0.01	no protection; depending on the case history basic immunization or boosting is necessary; serological control after 4-8 weeks
0.01 – 0.1	uncertain protection; boosting necessary; serological control after 4-8 weeks
0.11 – 0.5	protection only for a short time; boosting is recommended; boosting leads to long period protection
0.51 – 1.0	Protection exists; boosting or serological control is recommended after 3 years <b>advice: boosting with antibody concentrations &gt;0.5 IU/ml could lead to unwanted side effects!</b>
1.1 – 5.0	long term protection exists; boosting or serological control is recommended after 5 years
5.1 – 10.0	long term protection exists; boosting or serological control is recommended after 8 years
> 10.0	long term protection exists; boosting or serological control is recommended after 10 years

## 15. PERFORMANCE

### Recovery of spiked serum samples:

Deviation from the theoretical value is <8%.

### Intraassay variation (n=5-12):

based on the concentration: ≤12%.

### Interassay variation:

based on the concentration values within 0.11-0.5 IU/ml a CV of <26% was determined, at higher concentrations <10% (n=3).

### The lower detection limit:

0.09 IU/ml.

(Standards were calibrated on the WHO standard TE-3)

## 16. PRODUCT LITERATURE REFERENCES

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# Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di valutazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>Symbols of the kit components see MATERIALS SUPPLIED.  Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.  Voir MATERIEL FOURNI pour les symbôles des composants du kit.  Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.  Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.  Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.  Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.</p>	

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