*Instructions for Use*

# Thyroglobulin (Tg) ELISA

**Enzyme immunoassay for the quantitative detection of thyroglobulin (Tg) in human serum**

REF **RE70971**

Σ **96**

**2–8 °C**

EU: IVD CE

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

**I B L  I N T E R N A T I O N A L  G M B H**
Flughafenstrasse 52a
D-22335 Hamburg, Germany
Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11
IBL@IBL-International.com
www.IBL-International.com

# Instruction manual

## Contents

## 1. Intended Use

***Tg ELISA*** is an indirect solid phase enzyme immunoassay for the quantitative detection of thyroglobulin (Tg) in human serum. The assay employs selected specific monoclonal antibodies against human thyroglobulin (Tg).
The assay is a tool in the follow up and monitoring of thyroid carcinoma as well as for the differential diagnosis of thyroid diseases.

## 2. Clinical Application and Principle of the Assay

Thyroglobulin (Tg) is a glycoprotein of high molecular weight (660kDa) localized within the colloid of the thyroid follicle. It plays an essential role in the storage of iodine and acts as substratum for the synthesis of iodinated thyroid hormones thyroxine (T4) and 3,5,3`-triiodothyronine (T3).

Elevated thyroglobulin serum concentrations have been reported in various thyroid diseases, such as, hyperthyroidism, non-toxic goiter, thyroiditis and differenciated thyroid carcinoma.

The main indication of the Tg determination, however is the postoperative monitoring of differentiated thyroid carcinoma. Its clinical value is the early detection and exclusion of metastases or tumor relapses and the follow-up of radioiodine treatments. Serum Tg is non detectable in patients who underwent total thyroidectomy including ablation by radioiodine and are free of metastases and tumor. These patients in true complete remission will not display Tg levels, even by endogenous TSH stimulation.

Consequently detectable Tg values in these group of patients are an important indication for still existing or newly developed neoplasia. Particularly if these detectable Tg values are increasing under a TSH-suppressive thyroid hormone treatment (Tg profiles).

In contrast, thyroglobulin levels in patients with medullary carcinoma or undifferentiated tumors remain within the normal range. Since thyroglobulin levels could also be elevated in other benign thyroid diseases, this test is not a criteria for the diagnosis of malignant thyroid tumors.

Determination of thyroglobulin is of prognostic value in Graves` disease patients undergoing therapy.

Significantly elevated Tg levels at the end of a thyrostatic therapy are indication for a higher risk of relapse, whereas patients with continuous low thyroglobulin concentrations tend to continual recovery.

***Principle of the test***

Undiluted serum samples are incubated in the microplates coated with monoclonal antibodies against human thyroglobulin (Tg). Tg, if present in the specimen, bind to the antibodies. The unbound fraction is washed off in the following step. Afterwards monoclonal anti-Tg immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of Tg in the patient sample.

## 3. Kit Contents

***To be reconstituted:***

5x Sample Buffer 1 vial, 20 ml - 5x concentrated (capped white: yellow solution)
Containing: Tris, NaCl, BSA, sodium azide (preservative)

50x Wash Buffer 1 vial, 20 ml - 50x concentrated (capped white: green solution)
Containing: Tris, NaCl, Tween, sodium azide (preservative)

***Ready to use:***

Negative Control 1 vial, 1.5 ml (capped green: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)

Tg Recovery 1 vial, 3.0 ml (capped blue: yellow solution)
Containing: Thyroglobulin, sodium azide (preservative)

Control 10ng 1 vial, 1.8 ml (capped red: yellow solution)

Calibrators 5 vials, 1.5 ml each 0.5, 1.5, 5, 15, 50 ng/ml.
(color increasing with concentration: yellow solutions)
Exact Concentrations see vials lables.
Containing: Thyroglobulin, sodium azide (preservative)

Conjugate 1 vial,15 ml anti-Tg (capped white: green solution)
Containing: monoclonal anti-Tg antibodies conjugated to horseradish peroxidase

TMB Substrate 1 vial, 15 ml (capped black)
Containing: Stabilized TMB/$H_2O_2$

Stop Solution 1 vial, 15 ml (capped white: colorless solution)
Containing: 1M Hydrochloric Acid

Microtiterplate 12x8 well strips with breakaway microwells
Coating see paragraph 1

***Material required but not provided:***

Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass ware, test tubes for dilutions. Distilled water. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or multipipette. Microplate washing device (multichannel pipette or automated system), adsorbent paper.

## 4. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 4°C, at least. ***Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.***

## 5. Precautions of Use

### 5.1 Health hazard data

***THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY.*** Thus, only staff trained and specially advised in methods of in vitro diagnostics shall perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety :

***Recommendations and precautions***

This kit contains potentially hazardous components. Reagents may be irritating to eyes and skin thus avoid contact with eyes and skin and wear disposable gloves. Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth. All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases.

### 5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results. Allow all components to reach room temperature (20-26°C) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test. Always pipet substrate solution with clean tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

## 6. Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in dry tubes. After separation, the serum samples should be used immediately, respectively stored at 2-8°C up to three days, or frozen at -20°C for longer periods.

## 7. Assay Procedure

### 7.1 Preparations prior to pipetting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).
Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

**Washing**

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells
e.g. 4 ml concentrate plus 196 ml distilled water.

**Automated washing**:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

**Manual washing:**

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

**Microplates**

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C).

### 7.2 Work flow

***Patients samples have to be done twice, with and without addition of Tg Recovery.***

Pipette 100 µl of calibrators into the designated microwells.
Pipette 50 µl of negative control, Tg Recovery and patient`s sample into the designated wells.
Add 50 µl of sample diluent to negative control, Tg Recovery and patients samples for patients results without Recovery. Add 50 µl of Tg Recovery to patients samples for patients results with Recovery. Agitate plate carefully.
Incubate for 60 minutes at room temperature (20-26°C).
Wash 3x with 300 µl washing buffer (diluted 1:50).
Pipette 100 µl conjugate into each well.
Incubate for 60 minutes at room temperature (20-26°C).
Wash 3x with 300 µl washing buffer (diluted 1:50).
Pipette 100 µl TMB substrate into each well.
Incubate for 60 minutes at room temperature (20-26°C), in the dark.
Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate. Incubate 5 minutes minimum.
Agitate plate carefully for 5 sec.
Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

## 8. Quantitative Interpretation

Establish the standard curve by plotting the **optical density (O.D.) of each calibrator (y-axis)** with respect to the corresponding concentration values in **ng/ml (x-axis)**. For best results use linear regression with log-log coordinates for optical density and concentration (both logarithmic scale). From the O.D. of each sample, read the corresponding Tg concentrations expressed in **ng/ml.**

***Example of a standard curve***

We recommend pipetting calibrators in parallel for each run.

| *Calibrators Tg* | *O.D. 450/620 nm* | *CV %* |
|---|---|---|
| 0.5 ng/ml | 0.149 | 1.4 |
| 1.5 ng/ml | 0.246 | 2.6 |
| 5.0 ng/ml | 0.528 | 0.5 |
| 15.0 ng/ml | 1.049 | 1.3 |
| 50.0 ng/ml | 2.245 | 2.8 |

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations. ***Do not use this example for interpreting patients results!***

***Recovery test***

Anti-Tg antibodies or unspecific effects in a patient's serum may interfere with serum thyroglobulin assays. Consequently, sera should be tested for such interferences by carrying out a recovery test as follows.

In parallel to the original patient sample add 50 µl of Tg Recovery to 50 µl of the serum of investigation. Perform a Tg Recovery control (RC) by using 50 µl of sample diluent and 50 µl of Tg Recovery.

Recovery (in %) in the serum sample is calculated as stated below:

$$\frac{\text{ng Tg/ml (PR1)} - \text{ng Tg/ml (P1)}}{\text{ng Tg/ml (RC)}} \times 100 = \%\ \text{Recovery}$$

P1: Patient result without Tg recovery
PR1: Patient result with Tg recovery
RC: Tg Recovery control

Given unimpaired recovery (100%), (e.g. no factors are present in the patient‘s serum that interfere with Tg determination), the result shall be approximately 10 ng/ml above the Tg level of the corresponding original sample. Taking into consideration pipetting inaccuracies, recoveries between 70% and 130% are considered valid. Levels of < 70% or > 130% are due to interference and the Tg level of the relevant original sample has to be considered invalid.
The concentration for Tg Recovery is included in the enclosed quality control leaflet and is approximately 10 ng Tg/ml. Do not use QC data for calculation,

***Interpretation***
Positive results should be verified concerning the entire clinical status of the patient. Also every decission for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of serum Tg.

## 9. Technical Data

| | |
|---|---|
| **Sample material:** | serum |
| **Sample volume:** | 50 µl of sample, undiluted |
| **Total incubation time:** | 180 minutes at room temperature (20-26°C) |
| **Calibration range:** | 0.5 - 50 ng/ml |
| **Analytical sensitivity:** | 0.5 ng/ml |
| **Storage:** | at 2-8°C use original vials, only |
| **Number of determinations:** | 96 tests |

## 10. Performance Data

### 10.1 Functional assay sensitivity

The functional sensitivity of this kit has been found at 0.5 ng/ml.

### 10.2 Specificity

The microplate is coated with monoclonal antibodies highly specific for Tg. No crossreactivities to other antigens have been found.

### 10.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly.

### 10.4 Calibration

**Tg ELISA** is calibrated against the Certified Reference Material CRM 457 from BCR, Brussels for human Thyroglobulin. The results are expressed in ng/ml.

### 10.5 ”High dose hook” effect

Concentrations of up to 100,000 ng Tg/ml did not result in a ”high dose hook” effect.

### 10.6 Interference with autoantibodies

Chosen seras with various levels of anti-Tg have been spiked with Tg. No effect on results has been observed. However, this does not mean that all patient seras follow this results. Thus recovery test should be performed always.

## 11. Literature


**1. Gebel, F. et al.**
*The site of leakage of intrafollicular thyroglobulin into the blood stream in simple human goiter.*
J. Clin. Endocrinol. Metab. 1983; 57: 915 - 919.

**2. Uller, R.P. and van Herle, A.J.**
*Effect of therapy on serum thyroglobulin levels in patients with Graves` disease.*
J. Clin. Endocrinol. Metab. 1978; 46: 747 - 755.

**3. Gardner, et al.**
*Serum thyroglobulin in normal subjects and patients with hyperthyroidism due to Graves` disease: effects of T3, iodine, 131J, and antithyroid drugs.*
Clin. Endocr. (Oxf.) 1979; 11: 585 - 594.

**4. Kawamura, S. et al.**
*Serum thyroglobulin changes in patients with Graves` disease treated with long term antithyroid drug therapy.*
J. Clin. Endocrinol. Metab. 1983; 56: 507 - 512.

**5. Czernichow, P. et al.**
*Plasma thyroglobulin measurements help determine the type of thyroid defect in congential hypothyrodism.*
J. Clin. Endocrinol. Metab. 1983; 56: 242 - 245.

# Pipetting Scheme

| | | Calibrators (A-E) | Tg Recovery or Negative control | Samples | Samples with Tg Revovery |
|---|---|---|---|---|---|
| **Pipette** | Calibrators (A-E) | **100 µl each** | **-** | **-** | **-** |
| **Pipette** | Negative control | - | **50 µl each** | - | - |
| **Pipette** | Tg Recovery | **-** | **50µl each** | **-** | **50 µl each** |
| | serum samples | - | - | - | **50 µl each** |
| **Pipette** | sample diluent | **-** | **50 µl each** | **50 µl each** | **-** |
| **Pipette** | | | | | |
| Incubate | | ***60 min at room temperature (20-26°C)*** | | | |
| Decant | | ***Wash 3x with 300 µl of wash buffer (1x)*** | | | |
| **Pipette** | Conjugate | **100 µl** | | **100 µl** | **100 µl** |
| Incubate | | ***60 min at room temperature (20-26°C)*** | | | |
| Decant | | ***Wash 3x with 300 µl of wash buffer (1x)*** | | | |
| **Pipette** | Substrate | **100 µl** | | **100 µl** | **100 µl** |
| Incubate | | ***60 min at room temperature (20-26°C), in the dark.*** | | | |
| **Pipette** | Stop Solution | **100 µl** | | **100 µl** | **100 µl** |
| Incubate | | ***5 min at room temperature (20-26°C)*** | | | |

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

| | |
|---|---|
| REF | Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.: |
| LOT | 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: |
| CONC | Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα |
| LYO | Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο |
| IVD | 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 evaluazione. / 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: / Fabbricante: / Παραγωγός: |
|  | Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή! |

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.

## IBL AFFILIATES WORLDWIDE


| | | |
|---|---|---|
| **IBL International GmbH**<br>Flughafenstr. 52A, 22335 Hamburg, Germany | Tel.:<br>E-MAIL:<br>WEB: | + 49 (0) 40 532891 -0 Fax: -11<br>IBL@IBL-International.com<br>http://www.IBL-International.com |
| **IBL International B.V.**<br>Zutphenseweg 55, 7418 AH Deventer, The Netherlands | Tel.:<br>E-MAIL:<br>WEB: | + 49 (0) 40 532891 -0 Fax: -11<br>IBL@IBL-International.com<br>http://www.IBL-International.com |
| **IBL International Corp.**<br>194 Wildcat Road, Toronto, Ontario M3J 2N5, Canada | Tel.:<br>E-MAIL:<br>WEB: | +1 (416) 645 -1703 Fax: -1704<br>Sales@IBL-International.com<br>http://www.IBL-International.com |





Symbols Version 3.5 / 2011-07-01