ORGENTEC Diagnostika GmbH
Carl-Zeiss-Straße 49-51
55129 Mainz - Germany

Phone: +49 (0) 61 31 / 92 58-0
Fax: +49 (0) 61 31 / 92 58-58
Internet: www.orgentec.com

Instruction For Use
2014-01

CE

US Market: For Research Use Only

ORG 5TG Thyroglobulin

NAME AND INTENDED USE

Thyroglobulin is an ELISA test system for the quantitative measurement of thyroglobulin in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
Σ 96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Consult instructions for use
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	conform to European directive 98/79/EC

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
RECOVERY	Recovery
DILUENT	Sample Buffer
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
WASH	Stop solution
STOP	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly specific anti-human-thyroglobulin antibodies are bound to microwells.
The reaction is based on indirect enzyme immuno assay (ELISA) method with these steps: thyroglobulin present in a patient sample binds to the antibody coated forming an antigen-antibody-complex. Washing of the microwells removes unbound unspecific serum and plasma components. During incubation with enzyme-conjugate immunologically a conjugate/antibody/antigen complex is formed. Washing of the microwells removes unbound conjugate. An anti-thyroglobulin enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample. This assay includes a recovery test.

SUMMARY AND EXPLANATION OF THE TEST

Thyroglobulin (hTG) is a multiple glycosylated, water soluble iodoprotein. The molecular weight of appox. 660.000 Dalton is shared by two identical subunits. Thyroglobulin is synthesized in the thyrocytes of the thyroid gland and secreted into the lumen of the thyroid follicles. Iodination of the proteins tyrosyl residues lead to the precursors of the thyroid hormones T3 and T4. Finally the free T3 and free T4 are liberated into circulation, together with small amounts of thyroglobulin. Like for T3 and T4, synthesis and secretion of thyroglobulin is controlled by TSH and TRH. Suppressive medication using the thyroid hormones also leads to lower thyroglobulin serum concentration.
Elevated thyroglobulin serum concentrations have been reported in various thyroid diseases, such as

- hyperthyroidism,
- non-toxic goiter,
- thyroiditis,
- differenciated thyroid carcinoma.

Determination of thyroglobulin is a special prognostic value in Graves` disease patients undergoing therapy. Highly elevated hTG values at the end of a thyrostatic therapy are indicative for an early recidivation, whereas for patients with continuous low thyroglobulin concentrations prognosis tends to continual recovery.
A main application for the thyroglobulin determination is the post surgical monitoring of patients with differentiated thyroid carcinoma. After thyroidectomy, combined with x-ray therapy to destroy remaining thyroid tissue, one can expect an intermediate peak followed by a fast decrease of circulating thyroglobulin concentrations below the detection limit. Each renewed increase of serum thyroglobulin is indicative for residual thyroid tissue, a local recidivation of metastases. Due to its easy repeatability in the routine monitoring of thyroid carcinoma patients, the determination of thyroglobulin is a valueable non-invasive alternative and supplement to 131Iscintigraphy.

CONTENTS OF THE KIT

ORG 5TG	96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***THY***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 ng/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 3 ng/ml, containing thyroglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 10 ng/ml, containing thyroglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 30 ng/ml, containing thyroglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 100 ng/ml, containing thyroglobulin in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 300 ng/ml, containing thyroglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing thyroglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing thyroglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer STP, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow. Ready to use.
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human thyroglobulin antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
STOP	15 ml	Stop solution; contains acid. Ready to use.
RECOVERY	1x 3 ml	Recovery, 50 ng/ml, containing thyroglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1	Instruction for Use: ELISA Mini-DVD
	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 50 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, performe the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Sample buffer STP is ready to use.

Preparation of samples

Use undiluted sample. Note: Calibrators / Controls are ready to use and need no dilution.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **50 µl** of calibrators, controls and patient samples into the wells.
   Calibrators and controls: add **50 µl** sample buffer
   Patient samples: add **50µl** samples buffer (unspiked) / add 50µl RECOVERY (spiked)
   Incubate for **60 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **60 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
   The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1	P1+R									
B	B	P2	P2+R									
C	C	P3	P3+R									
D	D	P4	P4+R									
E	E	P5	P5+R									
F	F	P6	P6+R									
G	C+	P7	P7+R									
H	C-	P8	P8+R									

P1, ... patient sample (unspiked) P1+R, ... patient sample + RECOVERY A-F calibrators, C+, C- controls

RECOVERY Test

The presence of autoantibodies against thyroglobulin (anti-TG) can interfere with the determination of human thyroglobulin (hTG) in patient samples: anti-TG can attach to epitopes of hTG molecules and thus cause false negative results in the determination of hTG. Therefore, it is necessary to prove the presence of anti-TG autoantibodies in patient samples. This can be done either by direct quantitative measurement with an anti-TG test (e.g. ORG 503) or indirectly by recovery experiments in combination with the quantitative thyroglobulin determination.

In this Thyroglobulin assay a recovery test is included:

A patient sample is determined twice, unspiked and spiked with exogeneous hTG called **RECOVERY** which contains exactly 50 ng/ml hTG. The recovery test provides evidence as to the presence of anti-TG autoantibodies. Neither the correct anti-TG concentration nor the exact thyroglobulin concentration in presence of anti-TG can be calculated with this measurement.

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.

If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation. Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

The percentage recovery is calculated:

% recovery = (ng/ml hTG spiked / ng/ml hTG non-spiked + 50 ng/ml) * 100

Recovery should be expected in the range of 80-120 %.

If percentage of thyroglobulin recovery is below or above this range, thyroglobulin values for the respective patient sample should be excluded for further assessment.

PERFORMANCE CHARACTERISTICS

CALIBRATION

The assay system is calibrated against the international Certified Reference Material CRM 457 from BCR, Brussels for human Thyroglobulin.

Measuring range

The calculation range of this ELISA assay is 0 - 300 ng/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 2 - 50 ng/ml

Interpretation of results

According to literature cut-off values for serum thyroglobulin of around 60 ng/ml, with a median of 5 to 10 ng/ml. In newborn babies as well as in pregnant woman of the 3rd trimester higher thyroglobulin concentrations may be detected. For compehensive interpretation of thyroglobulin concentrations knowledge of alimentary iodine supply is indispensable. In regions with endemic goiter hTG values tend to be higher. In patients with total thyroidectomy no detectable thyroglobulin should be present after a xray therapy. Every increase of thyroglobulin to detectable serum concentrations is indicative for recidivation on thyroglobulin producing metastasis.

Linearity

Patient samples containing high levels of thyroglobulin were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

.

Sample	Dilution	Observed ng/ml	Expected ng/ml	O/E [%]
1	1:1	268.0	268.0	100
.	1:2	141.0	134.0	105
.	1:4	65.0	67.0	97
.	1:8	31.0	34.0	91
2	1:1	207.0	207.0	100
.	1:2	101.0	104.0	97
.	1:4	50.0	52.0	96
.	1:8	24.0	26.0	92

Limit of detection

Functional sensitivity was determined to be: 1 ng/ml

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample .	Mean ng/ml	. CV %
1	33.0	1.9
2	93.0	2.4
3	227.0	3.2

Inter-Assay		
Sample .	Mean ng/ml	. CV %
1	31.0	1.7
2	88.0	1.7
3	212.0	1.1

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually. The above pathological and normal reference ranges in patient samples should be regarded as recommendations only. Each laboratory should establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

REFERENCES

1. Marriq, S. et al. Polypeptide chains of 19-S thyroglobulin from several mammalian species and of porcine 27-S iodoprotein. Eur. J. Biochem. 1977; 79: 143 - 149.
2. Gärtner, D.F. et al. Evidence for autonomous thyroglobulin release from euthyroid and hyperthyroid nodular goiters - Thyroglobulin, a possible helpful parameter in diagnosis of non-malignant thyroid disorders. Klin. Wochenschr. 1983; 61: 737 - 741.
3. 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.
4. 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.
5. 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.
6. 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.
7. 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.
8. Mariotti, S. et al. Low serum thyroglobulin as a clue to the diagnosis of thyrotoxicosis factitia. New Engl. J. Med. 1982; 307: 410 - 412.
9. Reiners, C. Klinische Wertigkeit der Thyreoglobulinbestimmung im Serum. Akt. Endokr. Stoffw. 1984; 5: 76 - 82.
10. Kastrup, J. et al. An enzyme linked immunosorbent assay for measurement of human serum thyroglobulin. Evaluation of the influence of thyroglobulin autoantibodies. Scand. J. Lab. Invest. 1985; 45: 471 - 476.
11. Feldt-Rasmussen, U. et al. Serum thyroglobulin (Tg) in presence of thyroglobulin autoantibodies (TgAb). Clinical and methodological relevance of the interaction between Tg and TgAb in vitro and in vivo. J. Endocrinol. Invest. 1985; 8: 571 - 576.
12. van Herle, A.J. and Uller, R.P Elevated serum thyroglobulin: a marker of metestases in differentiated thyroid carcinomas. J. Clin. Invest. 1975; 56: 272 - 277.

Distributed By:
IBL-America, Inc.
8201 Central Ave NE, Suite P
Minneapolis, MN 55432, USA
info@ibl-america.com
(888) 523 1246