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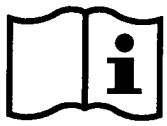

Instruction for use

Thyroglobulin (hTG) ELISA

Enzyme Immunoassay for quantitative determination of Thyroglobulin in human serum or plasma

CE

IVD

REF **DE7680**

Σ **96 Tests**

Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • D-24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
Email: info@demeditec.de • http://www.demeditec.com

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

Sufficient for 96 determinations

1 One divisible microplate consisting of 12 modules of 8 wells each. Ready to use.

6x 1.5 ml Calibrator A-F (0, 3, 10, 30, 100, 300 ng/ml), containing thyroglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use

2x 1.5 ml Control positive (1) and negative (2), containing thyroglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.

1x 3 ml Recovery, 50 ng/ml, containing thyroglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.

20 ml Sample Buffer STP, containing PBS, BSA, detergent, preservative NaN_3 0.09%, yellow. Ready to use

15 ml Enzyme Conjugate containing anti-human thyroglobulin antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use.

15 ml TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.

15 ml Stop Solution; contains acid. Ready to use.

20 ml Wash Solution, containing Tris, detergent, preservative NaN_3 0.09%; 50 x conc.

1 Instruction for Use

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 10 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 desiccated in the clip bag provided.
- Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash solution 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 solution.
- 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, classification is non-hazardous. Avoid contact with skin.
- Control, calibrator, sample buffer and wash solution contain sodium azide (NaN_3) 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 nitrile 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 Solution

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. 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.
2. Calibrators and controls: add **50 µ**l sample buffer
3. Patient samples: add **50µl** samples buffer (unspiked) / add **50µl** RECOVERY (spiked) Incubate for **60 minutes** at room temperature (20-28 °C).
4. Discard the contents of the microwells and **wash 3 times with 300 µl** of wash solution.
5. Dispense **100 µl** of enzyme conjugate into each well.
6. Incubate for **60 minutes** at room temperature.
7. Discard the contents of the microwells and **wash 3 times with 300 µl** of wash solution.
8. Dispense **100 µl** of TMB substrate solution into each well.
9. Incubate for **15 minutes** at room temperature
10. Add **100 µl** of stop solution to each well of the modules
11. Incubate for **5 minutes** at room temperature.
12. 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. DE7580) 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.

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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

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.

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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

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 establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
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	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
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Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • D-24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
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