

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

USA: PMS No. K952130

ORG 502 Anti-TG

NAME AND INTENDED USE

Anti-TG is an ELISA test system for the quantitative measurement of IgG class autoantibodies against thyroglobulin (TG) 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	MICROPLATE	Microplate
Manufacturer		CALIBRATOR A	Calibrator
REF	Catalogue number	CALIBRATOR B	Calibrator
96	Sufficient for 96 determinations	CALIBRATOR C	Calibrator
LOT	Batch code	CALIBRATOR D	Calibrator
Use by		CALIBRATOR E	Calibrator
Temperature limitation		CALIBRATOR F	Calibrator
Consult instructions for use		CONTROL +	Control positive
Keep away from sunlight		CONTROL -	Control negative
Do not reuse		DILUENT	Sample Buffer
Date of manufacture		CONJUGATE	Enzyme Conjugate
CE	conform to European directive 98/79/EC	TMB	TMB Substrate
		WASH	Stop solution
		STOP	Wash Buffer
		RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified human thyroglobulin (TG) is bound to microwells.

The determination is based on an indirect enzyme linked immune reaction with the following steps:

Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stops the reaction generating a yellow end-product. The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

SUMMARY AND EXPLANATION OF THE TEST

Thyroid disorders are the most prevalent of all autoimmune diseases. Thyroid autoimmune diseases are associated with the occurrence of differentiated autoantibodies and are thought to be related to a genetical pre-disposition. These autoantibodies are directed against membrane- located and/or extracellular antigens of the thyroid cells:

- Human Thyroglobulin (hTG), a water soluble glycoprotein with a molecular weight of approx. 660.000 Dalton, is the principal constituent of the thyroidal colloid sharing about 75 % of its mass. Synthesis of the thyroid hormones T3 and T4 is based on the oxidative iodination of tyrosine residues of the thyroglobulin molecule. Within the cell thyroglobulin is transported by the microsomes. Together with the secretion of T3 and T4 also small amounts of hTG are liberated into circulation.

- The microsomal antigen of the thyroid is an integral membrane protein of the microsomes. It has been characterised as the enzyme Thyroid Peroxidase (TPO) with a molecular weight of nearly 110.000 Dalton.-

The TSH-Receptor is a regulatory protein embedded into the thyroid cell membrane effecting synthesis and release of the thyroid hormones as well as cellular growth.

- The so-called Colloid-Antigen 2 CA2.

Besides these antibodies to functional antigens, antibodies directed against the circulating thyroid hormones T3 and T4 may occur. In Graves' Diseases -- an immunogenic form of hyperthyroidism -- often additional antibodies occur, which are directed against eye muscle antigens. They cause the endocrine ophthalmopathy.

Autoantibodies are found in inflammatory diseases as well as in thyroid autoimmune disorders. Various symptoms of thyroid diseases, like goitre, thyroid pain, hyperthyroidism and hypothyroidism may be caused by immunogenic processes and the occurrence of organ specific antibodies. This underlines the clinical relevance for autoantibody determination for the assessment of thyroid diseases.

The most important autoimmune diseases of the thyroid gland are: Hashimoto's Thyroiditis, Primary Myxedema, Graves' disease (often associated with endocrine ophthalmopathy) and other asymptomatic thyroid diseases, for example postpartum thyroiditis.

The simultaneous occurrence of Anti-TG and Anti-TPO autoantibodies seems to be related to their functional association. Tyrosine amino acid residues of the thyroglobulin molecule, as primary protein for the synthesis of the thyroid hormones T3 and T4, are actively iodinated in association with the thyroid peroxidase (TPO). TSH acts in stimulating synthesis and release of thyroid hormones in close cooperation of all the proteins. This makes the simultaneous appearance of all these antibodies plausible.

Persisting inhibition of the peroxidase activity by specific autoantibodies (Anti-TPO Abs) causes a decrease in the synthesis of thyroid hormones and thus hypothyroidism. Especially at the end of pregnancy, determination of thyroid antibodies may be a helpful diagnostic tool in the early diagnosis of an onset of post-partum hypothyroidism (Hashimoto's post partum depression). Hashimoto diseases are often associated with highly elevated titers of thyroid autoantibodies. The concentration of antibodies against thyroglobulin exceeds the titer of Anti-TPO antibodies, whereas in Graves' disease the opposite situation is found, with a stronger elevation of the Anti-TPO antibodies. Additionally, also high concentrations of TSH receptor antibodies are characteristic for both diseases.

Autoantibodies to

Disease (simplified)	TPO	TG	TSH receptor
Hashimoto Thyroiditis	xxx	xxx	x
Graves' disease (immunogenic)	xxx	x	xxx
Endocrine Orbitopathy	x	x	xx
Disseminated Autonomy (non immunogenic)	x	(x)	-
Regional Autonomy (autonomic Adenoma)	x	(x)	-

CONTENTS OF THE KIT

ORG 502	▽ 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: TG
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 IU/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow, yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 100 IU/ml, containing TG antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 300 IU/ml, containing TG antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 1000 IU/ml, containing TG antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 3000 IU/ml, containing TG antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 9000 IU/ml, containing TG antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing TG antibodies 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.
CONTROL -	1x 1.5 ml	Control negative, containing TG antibodies 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.
DILUENT	20 ml	Sample Buffer TG/TPO, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. 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.
i	1	Instruction for Use: ELISA Mini-DVD
i	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 by centrifugation.
- Test serum should be clear and non-hemolysed. Contamination by hemolysis or lipemia is best 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 samples. This may result in variable loss of autoantibody 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.
- Shelf life of the unopened 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, perform 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, classification 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 TG/TPO: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with

distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

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

- Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
Incubate for **30 minutes** at room temperature (20-28 °C).
Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
- Dispense **100 µl** of enzyme conjugate into each well.
Incubate for **15 minutes** at room temperature.
Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
- Dispense **100 µl** of TMB substrate solution into each well.
Incubate for **15 minutes** at room temperature
- 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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

P1, ... sample A-F calibrators C+, C- controls

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.

PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the international reference preparation WHO 65/93 for anti-thyroglobulin antibodies as 1000 IU/ml.

Measuring range

The calculation range of this ELISA assay is 0 - 9000 IU/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 150 IU/ml

Interpretation of results

Negative	< 100 IU/ml
Borderline	100 - 150 IU/ml
Positive	> 150 IU/ml

Linearity

Patient samples containing high levels of specific antibody 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 IU/ml	Expected IU/ml	O/E [%]
1	1:100	1366.9	1366.9	100
.	1:200	617.7	683.0	90
.	1:400	321.1	342.0	94
.	1:800	173.0	171.0	101
.	1:1600	84.9	85.0	99
2	1:100	3941.0	3941.0	100
.	1:200	1955.0	1971.0	99
.	1:400	998.0	985.0	101
.	1:800	499.0	493.0	101
.	1:1600	240.0	246.0	97

Limit of detection

Functional sensitivity was determined to be: 10 IU/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 IU/ml	CV %
1	746.0	2.6
2	1398.0	2.4
3	4674.0	5.0

Inter-Assay		
Sample	Mean IU/ml	CV %
1	765.0	5.7
2	1500.0	2.3
3	5565.0	4.0

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.

Study results

<u>Study population</u>	<u>n</u>	<u>n Pos</u>	<u>%</u>
Hashimoto's Thyroiditis, Graves'	130	125	96.2
Normal human sera	150	4	2.7

		Clinical Diagnosis	
		Pos	Neg
ORG 502	Pos	125	4
	Neg	5	146
		130	150

280

Sensitivity: 96.2 %
 Specificity: 97.3 %
 Overall agreement: 96.8 %

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 for antibodies 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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- 1 **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
 → **30 Minuten** bei Raumtemperatur inkubieren
 → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschlösung waschen
- 2 **100 µl** Enzymkonjugatlösung pipettieren
 → **15 Minuten** bei Raumtemperatur inkubieren
 → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschlösung waschen
- 3 **100 µl** Substratlösung pipettieren
 → **15 Minuten** bei Raumtemperatur inkubieren
- 4 **100 µl** Stopplösung zugeben
 → Platte **5 Minuten** stehenlassen
 → Bei **450 nm** messen