DRG® Thyroglobulin Ab (EIA-3708)

Revised 4 Jan. 2010 rm (Vers. 2.0) **RUO in the USA**

Please use only the valid version of the package insert provided with the kit.

NAME AND INTENDED USE

Anti-TG is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies against thyroglobulin (TG) in human serum or plasma. The assay is intended for in vitro use only as an aid in the diagnosis of Hashimoto´s Thyroiditis.

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:

- Thyroglobulin (hTg), a water soluble glycoprotein with a molecular weight of approx. 660.000 Dalton, is the principal constituent of the thyroidal colloid shareing about 75 % of it's mass. Synthesis of the thyroid hormones T3 and T4 is based on the oxidative iodination of thyrosine 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 characterized 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 und T4 may occur. In Graves' Diseases, an immunogenetic form of hyperthyroidism often additional antibodies occur, which are directed against eye muscle antigens. They cause the endocrine ophalmopathy.
Autoantibodies are found in inflammatory diseases as well as in thyroid autoimmune disorders. Various symptoms of thyroid diseases, like goiter, thyroid pain, hyperthyroidism and hypothyroidism may be caused by immunogenetic processes and the occurrence of organ specific antibodies. This underlines the clinical relevance for autoantibody determination for the assessment of thyroid diseases. Most important autoimmune diseases of the thyroid gland are:

- Hashimoto's Thyroiditis
- Primary Myxedema
- Graves' disease (often associated with endocrine ophtalmopathy)
- and other asymptomatic, for example postpartum thyroiditis.

The occurrence of anti-TG and anti-TPO autoantibodies at the same time seem to be related to their functional association. Thyrosine 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 Ab's) 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 is characteristic for both diseases.

The following table summarizes the distribution of autoantibodies in thyroid autoimmune diseases.

Disease (simplified)	Anti-TPO	Anti-TG	Anti TSH Receptor
Hashimoto Thyroiditis	XXX	XXX	X
Graves' disease (immunogen)	XX	X	XXX
Endocrine Orbitopathy	X	X	XX
Dissiminated Autonomy (non immunogen)	X	(X)	-
Regional Autonomy (autonom Adenoma)	X	(X)	-

PRINCIPLE OF THE TEST

Highly purified human thyroglobulin (TG) is bound to microwells. Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG antibodies present in the original sample.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro use only.
2. Do not interchange kit components from different lots.
3. 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.
4. Avoid contact with the TMB (3,3´,5,5´-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
5. Avoid contact with the Stop Solution which is acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.).

7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

CONTENTS OF THE KIT

Package size 96 determ.

Qty.1	Divisible **microplate** consisting of 12 modules of 8 wells each, coated with highly purified human thyroglobulin (TG). Ready to use.
6 vials, 1.5 ml each	combined **Calibrators** with IgG class Anti-TG antibodies (A-F) in a serum/buffer matrix (PBS, BSA, NaN_3 <0,1% (w/w)) containing: **IgG**: 100; 300; 1000; 3000; 9000 IU/ml Ready to use.
2 vials, 1,5 ml each	Anti-TG **Controls** in a serum/buffer matrix (PBS, BSA,NaN_3 <0,1% (w/w)) positive (1) and negative (2), for the respective concentrations see the enclosed QC Certificate. Ready to use.
1 vial, 20 ml	**Sample buffer** (Tris, NaN_3 <0.1% (w/w)), yellow, concentrate (5x).
1 vial, 15 ml	**Enzyme conjugate** solution (PBS, PROCLIN 300 <0.5% (v/v)), (light red) containing polyclonal rabbit **anti-human IgG**; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	**TMB substrate solution**. Ready to use.
1 vial, 15 ml	**Stop solution** (contains acid). Ready to use.
1 vial, 20 ml	**Wash solution** (PBS, NaN_3 <0.1% (w/w)), concentrate (50x).

STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8 °C.

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 µl

- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Data reduction software

Preparation of reagents
- Distilled or deionized water
- Graduated cylinder for 100 and 1000 ml
- Plastic container for storage of the wash solution

SPECIMEN COLLECTION, STORAGE AND HANDLING

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

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi quantitatively.

Revised 4 Jan. 2010 rm (Vers. 2.0) **RUO in the USA**

PREPARATION OF REAGENTS

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all patient samples **1:100** with sample buffer before assay.
Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well.

Controls are ready to use and need not be diluted.

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipet **100 µl** of calibrators, controls and prediluted patient samples in duplicate into the wells.
3. Incubate for 30 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 15 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 and incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Automation

The Anti-Phosphatidyl Serine IgG/IgM ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

Revised 4 Jan. 2010 rm (Vers. 2.0) **RUO in the USA**

INTERPRETATION OF RESULTS

Quality Control

This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Calibrator A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit ! If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

Calculation of results

For Anti-TG IgG a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Calculation example

The figures below show typical results for anti-TG ELISA. These data are intended for illustration only and should not be used to calculate results from another run.

Calibrators

No	Position	OD 1	OD 2	Mean	Conc. 1	Conc. 2	Mean	decl. Conc.	CV %
S1	A 1/A 2	0.028	0.029	0.028	1.7	1.7	1.7	0.0	0
S2	B 1/B 2	0.184	0.176	0.180	99	93	96	100	4
S3	C 1/C 2	0.452	0.433	0.443	318	300	309	300	4
S4	D 1/D 2	0.970	1.937	0.954	1016	955	986	1000	3
S5	E 1/E 2	1.571	1.555	1.563	3075	2979	3027	3000	1
S6	F 1/F 2	2.011	1.986	1.998	9370	8603	8986	9000	1

Interpretation of results

In a normal range study with 250 serum samples from healthy blood donors, regarding the 95and 98 percentile values, the following ranges have been established with the Anti-TG test kit:

	Anti-TG [IU/ml]
normal:	< 100
borderline:	100 - 150
elevated:	≥ 150

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges in serum. The above reference ranges should be regarded as guidelines only.

Revised 4 Jan. 2010 rm (Vers. 2.0) **RUO in the USA**

PERFORMANCE CHARACTERISTICS

Parallelism

In dilution experiments sera with high antibody concentrations were diluted with sample buffer and assayed in the Anti-TG kit.

Anti-TG	Sample	Dilution	Observed [IU/ml]	Expected [IU/ml]	O/E
IgG	1	1:200	1366.9		
		1:400	617.7	683.5	90 %
		1:800	321.1	341.5	94 %
		1:1600	142.8	170.8	84 %
IgG	2	1:200	1348.0		
		1:400	731.7	674.0	108 %
		1:800	358.7	337.0	106 %
		1:1600	201.0	168.5	119 %
		1:3200	100.0	84.3	118 %

Precision (Reproducibility)

Statistics for coefficients of variation (CV) were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations of each sample:

Intra-Assay		
Sample No	Mean [IU/ml]	CV [%]
1	746	2.6
2	1398	2.4
3	4674	5.0

Inter-Assay		
Sample No	Mean [IU/ml]	CV [%]
1	765	5.7
2	1500	2.3
3	5565	4.0

Sensitivity

The lower detection limit for Anti-TG ELISA was determined at 10 IU/ml.

Revised 4 Jan. 2010 rm (Vers. 2.0) **RUO in the USA**

Specificity

The microplate is coated with antigens, isolated from human thyroid tissue and highly purified by affinity chromatography. The Anti-TG test kit detects specifically IgG-class autoantibodies directed to the human thyroglobulin molecule.

Calibration

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

LIMITATIONS OF PROCEDURE

Not all Hashimoto´s Throiditis patients are positive for autoantibodies against TG.
The Anti-TG ELISA is an aid in the determination of the condition. 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.

INTERFERING SUBSTANCES

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

REFERENCES

1. Becker,W., Reiners,C., Börner,W. Immunologische Kriterien zur Differentialdiagnose von Schilddrüsenerkrankungen. Therapiewoche 1985;35:1167 - 1176.
2. Czarnocka,B., Ruf,J., Ferrand,M. et al. Purification of the human thyroid peroxidase and its identification as the microsomal antigen involved in autoimmune thyroid diseases.FEBSLett.1985;190:147 - 152.
3. Czarnocka,B., Ruf,J., Ferrand,M. et al. Interaction of highly purified thyroid peroxidase with anti-microsomal antibodies in autoimmune thyroid diseases.J.Endocrinol.Invest.1986;9:135 - 138.
4. Ruf,J., Czarnocka,B., Ferrand,M. et al. Thyroid peroxidase is the organ-specific 'microsomal' antigen involved in thyroid autoimmunity. Acta Endocrinol (Copenh) 1987;Suppl.281:49 -55.
5. Ruf,J., Czarnocka,B., Ferrand,M. et al. Relationship between immunological structure and biochemical properties of human thyroid peroxidase. Endocrinology 1988;Vol.125 (3):1211 - 1218.
6. Horster,F.A. Die Bedeutung von MAK,TAK,TRAK und Thyreoglobulin bei der Diagnose von Schilddrüsenkrankheiten. Internist 1988;29:538 – 540
7. Schatz,H., Löbig,H. Diagnostische und prognostische Bedeutung von Antikörpern gegen Schilddrüsenmikrosomen und Thyreoglobulin.Akt.Endokr.Stoffw.1989;10:146 - 153.
8. Hashimoto,H. Zur Kenntnis der lymphomatösen Veränderung der Schilddrüse (Struma lymphomatosa). Arch.Klin.Chir.1912;97:219 - 248.

9. Libert,F., Ruel,J., Ludgate,M.et al. Thyroperoxidase, an auto-antigen with a mosaic structure made of nuclear and mitochondrial gene modules. EMBO J.1987; 6:4193 - 4196.
10. McKenzie,J.M., Zakarija,M., Sato,A. Humoral immunity in Graves' disease. Clin. Endocrinol. Metab.1978;7:31 - 45.
11. Scherbaum,W.A., Berg,P.A. Bedeutung von Autoantikörpern in der Diagnostik endokrinologischer Erkrankungen. Dtsch.Med.Wschr.1981;106:308 - 313.
12. Scherbaum,W.A., Berg,P.A. Autoimmune Schilddrüsenerkrankungen - Neuere Aspekte zur Pathogenese und Diagnostik. Dtsch.Med.Wschr.1984;109:1574 - 1581.

Revised 4 Jan. 2010 rm (Vers. 2.0)

RUO in the USA

Symbols used with DRG 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-Konfirmitäts-kennzeichnung	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
Σ	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	Mindesthaltbarkeits-datum	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
Σ		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..