INSTRUCTION MANUAL

(April 01, 2014)

Medizym® Tg

- 96 determinations -

REF **3807**

Enzyme Immunoassay
for the determination of
Thyroglobulin (Tg) in human serum

MEDIPAN GMBH

Ludwig-Erhard-Ring 3

15827 Dahlewitz / Berlin (Germany)

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Phone: +49(0)33 708 / 44 17 - 0
Fax: +49(0)33 708 / 44 17 - 25

info@medipan.de
www.medipan.eu

IFU symbols non-radioactive assays MEDIPAN GMBH

RUO	For Research Use Only	LOT	Batch code
REF	Catalogue number		Manufactured by
	Expiry date		Consult operating instruction
	Consult accompanying documents		Biological risk
	Store at	CONJ	Conjugate
MP	Coated microtiterplate (96 wells)	SUB	Substrate
WASHB	Wash buffer	STOP	Stop solution
CAL	Calibrators	OD	Optical density
CONTROL	Control serum		

INTENDED USE

The Medizym® Tg is used for the quantitative and very sensitive determination of human thyroglobulin (hTg) in serum. Biochemically, Tg is to be understood as a rather complex family of molecules. It is microheterogeneous with inter- and intraindividual variations (iodination degree, carbohydrat contents etc.). Dimers and several fragments also exist. Additional heterogeneity is due to malignant de-differentiation. Specifically and unspecifically interfering factors in individual sera cause further problems. Therefore, the Tg determination still represents a rather ambitious method.

On the other hand, Tg is the substratum of the thyroid hormone synthesis. Only thyroid tissue (even of malignant nature, if still differentiated) has the ability to produce, to store and to secrete Tg. Consequently, Tg is organ- and tissue specific.

This is the basis for the main indication of the Tg determination**.** Its value consists in the early detection and exclusion of metastases or tumor relapses and in the reliable follow-up of radioiodine treatments. Tg-profiles are of particular meaningfulness. After total thyroidectomy (and ablation by radioiodine) **serum Tg is not detectable** in those who are free of metastases and tumor **(complete remission)**. Even under endogenous TSH stimulation, Tg normally remains undetectable.

Detectable Tg values, however, are well accepted as important indication for **neoplasia**. Of particular significance are Tg values which are already detectable on TSH-suppressive thyroid hormone treatment or which show a **steady increase** during this drug regimen **(Tg profiles)**. Another relevant criterion is a **significant Tg increase after thyroid hormone withdrawal**.

In the event, that any non-malignant thyroid remnants have been left, Tg is normally undetectable during TSH-suppressive thyroid hormone treatment. However, bigger remnants (> approx. 3 ml) or any co-existing non-malignant thyroid disease can lead in fact to detectable Tg. If the patient is on TSH stimulation, however, remnants as potential origin of measurable Tg have always to be taken into account.

In **benign thyroid diseases**, more or less elevated Tg values are regularly observed as compared to the reference range of healthy normal persons. Several factors (smoking, estrogenes, pregnancy, goitrogen drugs, iodine deficiency, TRAb etc.) and, in particular, disturbances of the **morphological integrity** of the gland (goitre, nodules, cellular destructions or thyroid autonomy etc.) act often complex and frequently lead to Tg elevations. Serum Tg is stimulated by TSH and is normally decreased by thyroid hormone administration (and iodine under certain circumstances, as well).

PRINCIPLE of the TEST

The Medizym Tg is a “sandwich” type of solid-phase enzyme immunoassay, based on two monoclonal antibodies that are specific for different epitopes of the Tg molecule (660 kDa). One of these antibodies is labeled with horseradish peroxidase (HRP) and acts as conjugate; the other is immobilized onto the surface of micotiter plates. Tg molecules from serum samples, calibrators and control bind to both immobilized antibodies and anti-Tg-peroxidase conjugate. Then the wells are washed with wash buffer to remove any material not bound to the inner surface of the wells. During the incubation with TMB substrate solution (3,3’,5,5’-tetramethylbenzidine) the blue coloring appears. The enzyme reaction is stopped by dispensing an acidic solution (1N HCl) into the wells, turning the solution from blue to yellow. Optical density (OD) of the solution at 405 nm resp. 450 nm is directly proportional to the amount of Tg bound. The standard curve is plotted by using the Tg concentrations of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The concentration of Tg of the specimen is directly read off from the standard curve.

If Tg values are above concentration of calibrator 6 (approx. 200 ng/ml), the sample has to be **diluted** with the Tg-free calibrator 0 (0 ng/ml). These samples should be analyzed again. In that way, higher Tg concentrations become accessible.

Tg measurement may be affected by presence of anti-Tg autoantibodies in the serum sample. In this case the results may be false-negative. To obtain reliable results it is recommended always to combine Tg and anti-Tg autoantibodies determination.

SAMPLE PREPARATION

Specimen collection and storage

Blood is taken by venipuncture. After clotting, the serum is separated by centrifugation. Do not use plasma, hemolysed or lipemic serum.

Store serum samples at 2 to 8 °C for no more than 3 days; for longer storage it is recommended to aliquote and freeze at -20 °C or below. Repeated freezing and thawing should be avoided.

Preparation before use

Prior to assay, allow the samples to reach room temperature. Take care to agitate serum samples gently in order to ensure homogeneity.

TEST COMPONENTS for 96 DETERMINATIONS

A MP	**Microtiter plate**, 12 breakable strips per 8 wells (total 96 individual wells) coated with anti-Tg antibodies (mouse, monoclonal)		1 platic bag
B WASHB	**Wash buffer** sufficient for 1000 ml solution		50 ml concentrate
D CONJ	**Conjugate** containing anti-Tg antibodies (mouse, monoclonal) coupled with HRP		14 ml ready for use
E SUB	**TMB Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide		14 ml ready for use
F STOP	**Stop solution** 1 N HCl solution		14 ml ready for use
0 - 6 CAL	**Tg calibrators** (protein-based buffer containing known Tg concentrations) conc.: see leaflet enclosed	L	**7 vials** Cal 0: 5 ml Cal 1-6: 1 ml ready for use
C CONTROL	**Tg control** (protein-based buffer containing known Tg concentrations) conc.: see leaflet enclosed	L	1 ml ready for use

Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 300 µl
- appropriate disposable tips
- microplate shaker-thermostat (able to maintain temperature +37°C and shaking speed 700 rpm; can be purchased from MEDIPAN)
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 405 nm, 450 nm and 620 nm or 690 nm
- graduated cylinders
- distilled or de-ionized water

Size and storage

Medizym® Tg has been designed for 96 determinations. This is sufficient for the analysis of 40 unknown samples as well as calibrators and control, assayed in duplicates.

The expiry date of each component is reported on its respective label, that of the complete kit on the box label.

Upon receipt, all components of the Medizym® Tg have to be kept at 2 to 8 °C, preferably in the original kit box.

Preparation before use

Allow all of the components to reach room temperature prior to use and stirre thouroughly before the assay.

A The microtiter plate is packaged in a plastic bag. The plate consists of a frame and strips with breakable wells. Before opening keep the bag at room temperature (18 to 25 °C) for 30 minutes. Open the bag and place required number of strips on strip holder. Put remaining strips back in plastic bag and close tightly. Keep at 2 to 8 °C until expiry date stated on the label.

B Prepare a sufficient amount of washing solution by diluting the concentrated wash buffer 20 times (1 + 19) with de-ionized or distilled water. For example, dilute 5 ml of the concentrate with 95 ml of water. Mix thoroughly, avoiding foaming. The diluted washing solution can be stored at 2 - 8 °C for 30 days.

The rest of the concentrated wash buffer should be stored firmly closed at 2 to 8 °C until expiry date.

E TMB Substrate: Avoid exposure of the substrate to light.

ASSAY PROCEDURE

Duplicates are recommended

1. Bring all reagents to room temperature before use. Mix gently without causing foam.
2. Dispense **100 µl** Conjugate (D) into the respective wells.
3. Dispense
   50 µl Tg calibrators (0 - 6)
   50 µl Tg control (C)
   50 µl neat serum
   into the respective wells.
4. Incubate **1 hour while shaking** (approx. 700 rpm) **at 37 °C**.
5. Decant, then wash each well 5 times using **300 µl** washing solution (prepared from B).
6. Add **100 µl** of TMB substrate (E) to each well.
7. Incubate **15 min while shaking** (approx. 700 rpm) **at 37 °C**.
8. Add **100 µl** of stop solution (F) to each well and mix gently for 5 sec.
9. Read the optical density at **450 nm and 405 nm** versus 620 nm or 690 nm within **15 min** after adding the stop solution.

DATA PROCESSING

Data processing is done by a computer assisted analysis calculating the mean OD values of calibrators 0 - 4 at 450 nm and 3 - 6 at 405 nm (see standardcurve) versus their respective Tg concentrations using spline smoothing fit.

Any extrapolation of the standard curve to Tg values above approximately 200 ng/ml (calibrator 6) is not permitted. Patients sera with such high Tg levels have to be diluted with the Tg-free calibrator 0 (0 ng/ml) provided. These samples have to be analyzed again. In that way, higher Tg concentrations become accessible.

TYPICAL EXAMPLE

Do not use for evaluation!

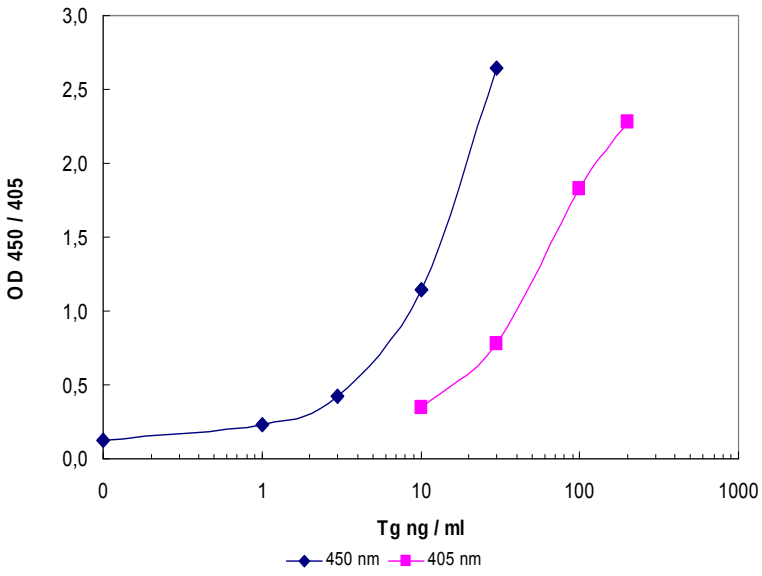
Sample	OD (a) 450 nm	OD (b) 450 nm	OD (mean)	ng Tg/ml
Calibrator **0**	0.121	0.120	0.121	**0**
Calibrator **1**	0.234	0.228	0.231	**1**
Calibrator **2**	0.415	0.434	0.425	**3**
Calibrator **3**	1.132	1.164	1.148	**10**
Calibrator **4**	2.643	2.643	2.643	**30**
Sample	1.712	1.749	1.730	**16.3**

Sample	OD (a) 405 nm	OD (b) 405 nm	OD (mean)	ng Tg/ml
Calibrator **3**	0.337	0.348	0.342	**10**
Calibrator **4**	0.783	0.781	0.782	**30**
Calibrator **5**	1.900	1.747	1.824	**100**
Calibrator **6**	2.319	2.249	2.280	**200**
Sample	0.506	0.514	0.510	**16.4**

The above mentioned standard concentrations are only an example for a typical standard curve. They can change from lot to lot.

STANDARD CURVE

Typical example



REFERENCE VALUES

Normal values

Approximately 2 - 70 ngTg/ml (median approx. 13 ng/ml), slightly lower in areas of sufficient iodine supply (approx. 1 - 35 ng/ml, median approx. 10 ng/ml).

It is recommended that each laboratory establishes its own normal and abnormal reference ranges for serum Tg levels, as usually done for other parameters. Therefore, the above mentioned data only provide a guide to values which might be expected.

CHARACTERISTICAL ASSAY DATA

Calibration

Medizym® Tg is 1:1 calibrated against the International Tg Reference Material CRM 457 (Community Bureau of Reference, BCR, European Union, Brussels, Belgium).

Parallelism of serum samples

Human sera of high Tg contents lead to the expected results within the usual margins of error after appropriate serial dilution with Tg-free human serum. When using the Tg-free calibrator 0 (0 ng Tg/ml), equivalent data are observed.

Specificity

The falsification of any Tg determination by specifically (anti-Tg) and unspecifically acting serum factors can in principle not be excluded.

Both monoclonal antibodies used for kit development show no cross-reactivity with triiodothyronine (T_3) or thyroxin(T_4) and could not be deteced even in supra-physiological concentrations.

Medizym® Tg shows with Tg concentration up to 100.000 ng/ml no **high dose hook effect**. However, Tg concentrations above about 100.000 ng/ml result in OD values below calibrator 6 and resulting Tg values become consequently more and more falsely low, if the original serum is measured undiluted. In case of such a phenomenon serum should be re-tested at 1:100 predilution.

Sensitivity (lower detection limit)

The analytical sensitivity is 0.3 ng Tg/ml calculated as mean (n = 12) of Tg-free serum +3 SD.

The most appropriate and statistically reasonable definition of the lower detection limit of any assay is at present the so-called **functional assay sensitivity**.

The functional assay sensitivity generally represents that concentration, which corresponds to the 20 % (between-assay) coefficient of variation in the respective precision profiles of the assay in the lower concentration range. Upon correct and thorough performance of the Medizym® Tg, this value is at approx. 1.9 ngTg/ml (1:1 CRM 457).

Formally determined Tg values below this Tg level do not meet the statistical criteria for reliability according to GLP (Good Laboratory Practice) and can, therefore, not be distinguished from zero with the statistically necessary certainty.

Tg concentrations above approx. 1.9 ng/ml, however, fulfill these criteria and are consequently assessed as valid.

Intra- and inter- assay variation

Intra-assay			Inter-assay		
Sample no.	Mean Concentration (ng/ml)	CV (%)	Sample no.	Mean Concentration (ng/ml)	CV (%)
1	1.0	13	4	0.9	25
2	4.4	3	5	3.2	10
3	13.6	3.5	6	17.0	5

Medizym® Tg

ASSAY SCHEME

Step	Activity	Material	CAL 0-6	CONTROL (C)	Sample 1, 2…
1	Pipette	Conjugate (D)	100 µl	100 µl	100 µl
2	Pipette	Calibrators 0-6 Control (C) Patient samples	50 µl	50 µl	50 µl
3	Incubate	Plate	**1 hour shaking at +37 °C (approx. 700 rpm)**		
4	Wash	Washing solution made from B	5 x 300 µl	5 x 300 µl	5 x 300 µl
5	Pipette	TMB Substrate (E)	100 µl	100 µl	100 µl
6	Incubate	Plate	**15 min shaking at +37 °C in the dark (approx. 700 rpm)**		
7	Pipette	Stop solution (F)	100 µl	100 µl	100 µl
8	Stirring	Plate	**5 sec shaking (approx. 700 rpm)**		
9	Measure OD **at 450 nm and 405 nm** against 620 nm (690 nm) **within 15 min**				

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. This instruction manual is valid only for the present kit with the given composition. An exchange of single components is not in agreement with CE regulations.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Kathon MW as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

In any case GLP should be applied with all general and individual regulations to the use of this kit.