# INSTRUCTION MANUAL

IVD

( February 1st, 2008 )

# Medizym® Tg Rem

**- 96 determinations -**

REF **4018**

CE

**Highly sensitive ELISA**
for the determination of **Thyroglobulin** (Tg)
in human serum


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**IFU symbols non-radioactive assays MEDIPAN GMBH**

| | | | |
|---|---|---|---|
| IVD | In vitro diagnostic device | CE | EC Declaration of Conformity |
| REF | Catalogue number | LOT | Batch code |
| | Expiry date | | Manufactured by |
| | Consult accompanying documents | | Consult operating instruction |
| | Store at | D | Biological risk |
| MP | Coated microtiterplate (96 wells) | OD | Optical density |
| WASHB | Wash buffer | SUB | Substrate |
| CAL | Calibrators | CONJ | Conjugate |
| STOP | Stop solution | CONTROL | Control serum |
| BUF D | Conjugate diluent | START | Incubation buffer |
| REC | Recovery test sample | DIL | Sample diluent |

## INTENDED USE

**The Medizym® Tg Rem is an Enzyme-linked immunosorbent assay used for the quantitative and very sensitive determination of human thyroglobulin (hTg) in serum especially designed for the follow up of patients with differentiated thyroid cancer in remission.**

Biochemically, Tg is to be understood as a rather complex family of molecules. It is micro heterogeneous with inter- and intra-individual variations (iodination degree, carbohydrate 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 **(postoperative monitoring of differentiated thyroid carcinoma).** Its paramount clinical 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 anymore** in patients 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 still existing or newly developed **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 (goiter, 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 Rem is an immunoenzymometric assay (IEMA). One of the two specially selected monoclonal anti-Tg antibodies is immobilized onto the surface of microtiter plates. The other one is horseradish peroxidase (HRP) labeled and acts as conjugate. Both monoclonals are used in excess and bind Tg at different epitopes, which are relatively free from interferences.

Tg of serum samples, controls and calibrators react with the solid phase bound anti-hTg antibody during the first incubation period of 2.5 hours at room temperature. After removal of the non-bound components by a washing step anti-hTg HRP conjugate interacts with the Tg molecules trapped by the immobilized anti-hTg antibody during the second incubation at room temperature over night (> 15 hours). The sandwich type complexes formed are separated from unbound material by a further washing step. HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution ($H_2SO_4$) into the wells after 15 min, turning the solution from blue to yellow.

The optical density (OD) of the solution at 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 approx. 3 ng/ml, the sample has to be **diluted** 1 in 100 with the Tg-free serum diluent provided. These samples are again analyzed. In that way, concentrations up to 300 ngTg/ml become accessible. It is recommended to analyze the serum diluent G as unknown sample 1, representing the Tg-negative control. Its corresponding recovery sample GR serves as control for the 100 % value of the recovery experiment.

# PATIENTS SAMPLES

## Specimen collection and storage

Blood is taken by venipuncture. After clotting, the serum is separated by centrifugation. Lipaemic and hemolytic samples or plasma should not be employed.

The samples may be kept at 2 - 8 °C for up to three days. For long-term storage, - 20 °C are necessary. Repeated freezing and thawing should be avoided. If required, the samples have to be initially frozen in aliquots.

## 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 h-Tg antibodies (mouse, monoclonal) | | 1 vacuum sealed with desiccant |
| **B** WASHB | **Wash buffer** sufficient for 1250 ml solution | | 125 ml concentrate |
| **G** DIL | **Sample diluent** | | 100 ml ready for use |
| **D** CONJ | **Conjugate** containing h-Tg antibodies (mouse, monoclonal) coupled with HRP | | for 20 ml lyophilized |
| **J** BUF D | **Conjugate diluent** | | 25 ml ready for use |
| **E** SUB | **TMB Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide | | 12 ml ready for use |
| **F** STOP | **Stop solution** 0.25 M sulfuric acid | | 12 ml ready for use |
| **H** START | **Mouse IgG** | | 4 ml ready for use |
| **R** REC | **Recovery test sample** (serum diluted) 5 ng Tg/ml | | 1.5 ml lyophilized |
| **0** CAL | **Tg calibrator** (serum Tg-free) conc.: see leaflet enclosed | L | 1 ml Ready for use |
| **1 - 8** CAL | **Tg calibrators** (serum diluted) conc.: see leaflet enclosed | L | 1 ml lyophilized |
| **CI - CII** CONTROL | **Tg control sera** (human serum) conc.: see leaflet enclosed | L | 1 ml lyophilized |

## Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- graduated cylindersdistilled or de-ionized water

## Size and storage

Medizym® Tg Rem has been designed for 96 determinations. This is sufficient for the analysis of 19 unknown samples with their respective recovery tests as well as calibrators and control sera, 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 Rem have to be kept at 2 - 8 °C, preferably in the original kit box.

## Preparation before use

Allow all of the components to reach room temperature prior to use in the assay.

A The microtiter (A) plate is vacuum sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening for at least 30 minutes. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

B Prepare a sufficient amount of washing solution by diluting the concentrated wash buffer (B) 10 times (1 + 9) with de-ionized or distilled water. For example, dilute 20 ml of the concentrate with 180 ml of distilled water. The washing solution prepared is stable at 2 - 8 °C up to 30 days.

C Reconstitute the lyophilized calibrators and controls by adding 1 ml distilled water. Reconstituted calibrator and control solutions are stable at - 20 °C up to three months. Dilute reconstituted controls 1 in 10 with diluent (G) prior to use. For example, dilute 20 µl of the control with 180 µl sample diluent (G).

D Reconstitute the lyophilized conjugate (D) by adding 20 ml of conjugate diluent (J). The reconstituted conjugate solution is stable at 2 - 8 °C up to three months.

E Avoid exposure of the substrate to light.

R For recovery calculation serum samples have to be spiked with 10 µl recovery test sample. Reconstitute the lyophilized recovery test sample (R) by adding 1.5 ml distilled water and dilute it in sample diluent (G) 4-fold (1.0 ml + 3.0 ml). This reconstituted sample is stable at 2 - 8 °C up to 3 months.

# ASSAY PROCEDURE

- **Duplicates are recommended.**

1. Bring all reagents to room temperature before use. Mix gently without causing foam.
2. Dispense 25 µl mouse IgG (H) into the respective wells.
3. Dispense
   25 µl calibrators (alternatively plus calibrator 8)
   25 µl controls (CI/II) diluted 1 in 10 or 1 in 100
   25 µl sample diluent (G) + 10 µl recovery sample (R)
   25 µl neat patient serum
   25 µl corresponding serum + 10 µl recovery sample.
   into the respective wells.
4. Incubate 2.5 hours while shaking at room temperature (18 - 25 °C).
5. Decant, then wash each well three times using 300 µl washing solution (prepared from B).
6. Add 150 µl of conjugate solution (prepared from D and J) to each well.
7. Incubate over night (> 15 h) at room temperature (18 - 25 °C).
8. Decant, then wash each well three times using 300 µl washing solution.
9. Add 100 µl of substrate (E) to each well.
10. Incubate 15 min *in the dark* at room temperature (18 - 25 °C).
11. Add 50 µl of stop solution (F) to each well and shake gently.
12. Read the optical density at 450 nm versus 620 or 690 nm within 15 min after adding the stop solution.

# DATA PROCESSING

This data processing is done by a computer assisted analysis calculating the mean OD values of calibrators 1 - 7 versus their respective Tg-concentrations using spline smoothing fit.

Any extrapolation of the standard curve to Tg values above 3 ng/ml (calibrator 7) is not permitted. Patients sera with such high Tg levels have to be diluted 1 : 100 (alternatively 1 : 500) with the Tg-free sample diluent (G) provided. These samples have to be analyzed again (including recovery tests). In this way, values up to 300 and 1.500 ngTg/ml, become available, respectively.

In addition calibrator 8 (10 ng/ml) can be used for higher concentrations, however, measuring the optical densities at 405nm instead of 450nm. Thus concentrations between 1.0 and 10 ng/ml can be determined.

# TYPICAL EXAMPLE

**Do not use for evaluation!**

| Well | OD (a) 450 nm | OD (b) 450 nm | OD (mean) | ngTg/ml |
|---|---|---|---|---|
| Calibrator **0** | 0.084 | 0.076 | 0.080 | **0.0** |
| Calibrator **1** | 0.141 | 0.151 | 0.146 | **0.03** |
| Calibrator **2** | 0.193 | 0.207 | 0.200 | **0.05** |
| Calibrator **3** | 0.330 | 0.314 | 0.322 | **0.1** |
| Calibrator **4** | 0.739 | 0.721 | 0.730 | **0.3** |
| Calibrator **5** | 1.815 | 1.785 | 1.800 | **1.0** |
| Calibrator **6** | 2.608 | 2.638 | 2.623 | **2.0** |
| Calibrator **7** | 3.081 | 3.155 | 3.118 | **3.0** |
| Control **I** | --- | --- | --- | --- |
| Control **II** | --- | --- | --- | --- |
| Patient P1 | 0.062 | 0.068 | 0.064 | **< 0.03** |
| Patient P1R | 0.994 | 0.963 | 0.979 | **0.44** |

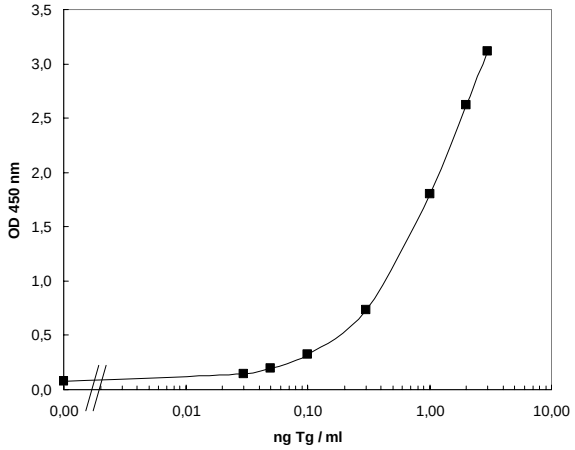
## Calculation of the recovery

$$\frac{P1R - P1}{GR} \times 100 = \%\ \text{Recovery of Patient 1}$$

All values are in ngTg/ml. GR is the recovery sample in ngTg/ml of the Tg-free sample diluent G and recommended for the calculation instead of the theoretical value. The theoretical value of GR is 0.5 ngTg/ml.

# STANDARD CURVE

**Typical example**




# REFERENCE VALUES

- **Healthy normal persons:**
  approx. 2 - 70 ngTg/ml (median approx. 13 ng/ml),
  slightly lower in areas of sufficient iodine supply
  (approx. 1 - 35 ng/ml, median at approx. 10 ng/ml).

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

# CHARACTERISTICAL ASSAY DATA

## Calibration

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

## Parallelism of Calibrators and serum samples

Defined dilutions of the reference material in human serum (Tg-free) measured in the Medizym® Tg show results as expected. Human sera of high Tg contents also 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 sample diluent G, 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. Therefore, every Tg value has to be checked for accuracy by means of the **recovery test**: **Parallel determination** of the sample in the same assay after a known amount of Tg has been added.

In case of un-interfered recovery (100 %) in the Medizym® Tg Rem, the Tg value of the respective recovery test sample is approx. 0.5 ngTg/ml higher than that of the corresponding original sample. Routinely, recovery results between 60 and 140 % are assessed as correct. In contrast, values below 60 % and above 140 % represent incorrect recovery and normally indicate a falsely-low Tg value in the corresponding original serum.

In order to check the basic reliability of the performance of that test, it is recommended to run the recovery test for the sample diluent G, as well. The result of the sample GR shows the experimental 100 % value. The sample G itself simultaneously represents the Tg-negative control.

Any **cross reactivity** with thyroxin or tri-iodothyronine and HSA, respectively, could not be detected even in supra-physiological concentrations.

**Falsely-negative determinations due to a high-dose-hook effect are excluded because of the consecutive incubation of serum and conjugate.**

## Sensitivity (lower detection limit)

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 Rem, this value is at approx. 0.03 ngTg/ml.

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. 0.03 ng/ml, however, fulfill these criteria and are consequently assessed as valid.

# LIMITATIONS of the METHOD

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

# Medizym® Tg Rem

## ASSAY SCHEME

| Step | Activity | Material | CAL | CI - CII | GR | Pat. 1, 2… | P1R, P2R, .. |
|---|---|---|---|---|---|---|---|
| 1 | Pipette | Mouse IgG (H) | 25 µl | 25 µl | 25 µl | 25 µl | 25 µl |
| 2 | Pipette | Calibrators*<br>CI, CII (1/10 dil.)<br>Sample diluent (G)<br>Patient sample<br>Recovery test sample (R) | 25 µl | 25 µl | 25 µl<br>10 µl | 25 µl | 25 µl<br>10 µl |
| 3 | Incubate | Plate | 2.5 hours shaking at room temperature (18 - 25 °C) | | | | |
| 4 | Wash | Washing solution | 3 x 300 µl | 3 x 300 µl | 3 x 300 µl | 3 x 300 µl | 3 x 300 µl |
| 5 | Pipette | Conjugate (D) | 150 µl | 150 µl | 150 µl | 150 µl | 150 µl |
| 6 | Incubate | Plate | Over night (15 hours minimum) at room temperature | | | | |
| 7 | Wash | Washing solution | 3 x 300 µl | 3 x 300 µl | 3 x 300 µl | 3 x 300 µl | 3 x 300 µl |
| 8 | Pipette | Substrate (E) | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl |
| 9 | Incubate | Plate | 15 min at room temperature in the dark | | | | |
| 10 | Pipette | Stop solution (F) | 50 µl | 50 µl | 50 µl | 50 µl | 50 µl |
| 11 | Measure OD at 450 nm against 690 (620) nm within 15 min, if necessary at 405 nm | | | | | | |

* Calibrator 8 (10 ngTg/ml) optional

## 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 (< 0.1 % w/v) of sodium azide and chloroacetamid-N-methylisothiazolone as preservatives. 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.