



# INSTRUCTION MANUAL

IVD

( May 30<sup>th</sup>, 2008 )

# Medizym<sup>®</sup> TRAb clone

- 96 determinations -

European Patent  
EP 1021721

REF 3805



ELISA for the determination of autoantibodies to TSH receptor in human serum using a **human stimulating** monoclonal antibody



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## IFU symbols non-radioactive assays MEDIPAN GMBH

<b>IVD</b>	In vitro diagnostic device		EC Declaration of Conformity
<b>REF</b>	Catalogue number	<b>LOT</b>	Batch code
	Expiry date		Manufactured by
	Consult accompanying documents		Consult operating instruction
	Store at	<b>D</b>	Biological risk
<b>MP</b>	Coated microtiterplate (96 wells)	<b>OD</b>	Optical density
<b>WASHB</b>	Wash buffer	<b>SUB</b>	Substrate
<b>CAL</b>	Calibrators	<b>CONJ</b>	Conjugate
<b>STOP</b>	Stop solution	<b>CONTROL</b>	Control serum
<b>M22</b>	M22 biotin complex	<b>START</b>	Incubation buffer
<b>BUF D</b>	Conjugate diluent		

## INTENDED USE

Medizym<sup>®</sup> TRAb clone is used for the quantitative determination of Thyrotropin (TSH) receptor autoantibodies in human serum.

Whereas thyrotoxicosis is caused by thyroid autonomy, hyperthyroidism of Graves' disease is due to TSH receptor autoantibodies (TRAb). These autoantibodies mimic TSH effects on the thyroid cell and thus increase blood levels of T<sub>4</sub> and T<sub>3</sub>.

Consequently, the measurement of TRAb is valuable for the differential diagnosis of hyperthyroidism as well as for the follow-up of Graves' disease, both during and after its treatment by antithyroid drugs, 131-radioiodine or surgery. Possible additional diagnostic relevance has to be established in the future.

## LITERATURE

- Kakinuma A, Morimoto I, Kuroda T, Fujihira T, Eto S, McLachlan SM, and Rapoport B. Comparison of recombinant human thyrotropin receptors versus porcine thyrotropin receptors in the thyrotropin binding inhibition assay for thyrotropin receptor autoantibodies. Thyroid 1999; 9 (9): 849 – 855.
- Kamijo K. TSH-receptor antibody measurement in patients with various thyrotoxicosis and Hashimoto's Thyroiditis: a comparison of two two-step assays, coated plate ELISA using porcine TSH-receptor and coated tube radioassay using human recombinant TSH-receptor. Endocrine J 2003; 50 (1): 113 – 116.

## PRINCIPLE of the TEST

Medizym<sup>®</sup> TRAb clone is a competitive enzyme immunoassay with a step by step incubation.

During the first incubation the TSH receptor antibodies of the patient samples and calibrators bind to the receptor immobilized on the solid phase of the microtiter plate. Following an incubation period of 120 min, antibodies not bound are separated from the solid-phase immune complexes. In a second incubation step of 25 min the M22 stimulating monoclonal human antibody biotin complex binds to the free epitopes of the receptor and / or is going into competition with the autoantibodies bound.

The absence of autoantibodies against TSH receptor results in a complete saturation of the provided receptor by the M22 complex. Thus, the more autoantibodies (TRAb) are present in the sample, the less complex is bound by the TSH receptor immobilized.

The bound receptor-M22 antibody complexes then react specifically with streptavidin peroxidase (SA-POD) conjugate. After the incubation period of 20 min, excessive conjugate is separated from the solid-phase immune complexes by the following washing step.

Horseradish peroxidase converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. This enzyme reaction is stopped by dispensing an acidic solution (H<sub>2</sub>SO<sub>4</sub>) into the wells after 30 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is indirectly proportional to the amount of specific antibodies bound.

The standard curve is established by plotting the concentrations of the antibodies of the standards (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve.

## PATIENT SAMPLES

### Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Do not use lipaemic or hemolytic samples. Plasma should not be employed. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

### Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C.

**Note:** Before assayed the sera have to be free of any particulate matter (centrifuge, if necessary and use the **clear supernatants** only).

## TEST COMPONENTS for 96 DETERMINATIONS

<b>A</b>	<b>Microtiter plate</b> , 12 breakable strips per 8 wells (total 96 individual wells) coated with TSH receptor		1 frame vacuum sealed with desiccant
<b>MP</b>			
<b>B</b>	<b>Wash buffer 10-fold</b> for 1000 ml		100 ml concentrated
<b>WASHB</b>			
<b>G</b>	<b>Human M22 biotin complex</b>		15 ml ready for use
<b>M22</b>			
<b>D</b>	<b>SA-POD conjugate for 15 ml</b>		1 x 0.75 ml concentrated
<b>CONJ</b>			
<b>J</b>	<b>Conjugate diluent</b>		15 ml ready for use
<b>BUF D</b>			
<b>E</b>	<b>Substrate TMB</b> 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide		15 ml ready for use
<b>SUB</b>			
<b>F</b>	<b>Stop solution</b> 0.25 M sulfuric acid		10 ml ready for use
<b>STOP</b>			
<b>H</b>	<b>Incubation buffer</b>		10 ml ready for use
<b>START</b>			
<b>1 - 5</b>	<b>TRAb calibrators</b> (serum) conc.: see leaflet enclosed	┌	1.0 ml, each ready for use
<b>CAL</b>			
<b>CI-CII</b>	<b>Controls</b> (serum) conc.: see leaflet enclosed	┌	1.0 ml, each ready for use
<b>CONTROL</b>			

### Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl trough for multi-channel pipette
- 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
- horizontal shaker
- graduated cylinders
- very pure distilled water
- foil to seal microtiterplate

### Size and storage

Medizym® TRAb clone has been designed for 96 determinations. This is sufficient for the analysis of 41 unknown samples as well as for calibrators and controls assayed in duplicates.

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

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

### Preparation before use

Allow sera to reach room temperature prior to use in the assay.

The kit includes the frame for the microtiter plate.

Allow the sealed plate to reach room temperature (20 - 25 °C) for at least 30 min before opening.

#### A Microtiter plate

After opening a bag of strip wells keep any unused wells in the foil packet (reseal with adhesive tape) and place in the bag provided with desiccant. Wells can be stored this way at 2 - 8 °C up to 12 weeks.

#### B Wash buffer

Prepare a sufficient amount of washing solution by diluting the concentrated wash buffer (B) 1 + 9 with distilled water. For example, dilute 50 ml of the concentrate with 450 ml distilled water. B should be free of crystals before dilution, otherwise dissolve by warming up to max. 37 °C. The diluted washing solution can be stored at 2 - 8 °C up to 30 days.

#### D SA-POD conjugate

Dilute streptavidin-peroxidase concentrate (D) 20-fold (1+19) with conjugate diluent (J) **prior to use**.

For example, 0.5 ml SA-POD concentrate + 9.5 ml conjugate diluent = 10 ml conjugate ready for use. For 96 determinations: 96 x 100 µl = 9.6 ml conjugate are necessary.

The diluted SA-POD conjugate is stable at 2 - 8 °C up to 4 weeks.

#### E Substrate TMB

Avoid exposure to light!

## ASSAY PROCEDURE

- Use neat patient sera
- Duplicates are recommended

1. Pipette **75 µl** incubation buffer (H) in the provided wells.
2. Dispense **75 µl** control (CI) **75 µl** calibrators (1 - 5) **75 µl** controls (CII) **75 µl** neat patient samples into the respective wells, shake for 5 sec.
3. Seal plate and incubate **120 min** at room temperature (20 - 25 °C) while shaking vigorously (>500 rpm). An increase of the incubation time to 160 min will improve the accuracy of the standard curve. Without any shaking the incubation time is at least 180 min.
4. Aspirate or "flick out" by striking the wells sharply onto absorbent paper to remove any residual droplets. Wash once with 300 µl washing solution (diluted from B) with 5 seconds soaking time.
5. Add **100 µl** of M22 biotin complex to each well, shake for 5 sec.
6. Seal plate, incubate **25 min** at room temperature (20 - 25 °C) without shaking.
7. Repeat step 4.
8. Add **100 µl** of diluted conjugate (prepared from D and J) to each well, shake for 5 sec.
9. Seal plate, incubate **20 min** at room temperature (20 - 25 °C) without shaking.
10. Aspirate or "flick out" by striking the wells sharply onto absorbent paper to remove any residual droplets. Wash 3 times with 300 µl washing solution (diluted from B) with 5 seconds soaking time each.
11. Add **100 µl** of substrate (E) to each well, shake for 5 sec.
12. Incubate **30 min** in the dark at room temperature (20 - 25 °C).
13. Add **50 µl** of stop solution (F) to each well after exactly 30 min for each single well and mix gently.
14. Read the optical density at **450 nm** versus 620 or 690 nm within 20 min after adding the stop solution.

Please note that the washing procedure is crucial. Insufficient washing will result in poor precision and falsely elevated OD readings.

## DATA PROCESSING

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 5 on the ordinate, y-axis, versus their respective TRAb-concentrations on the abscissa, x-axis. In addition negative control (CI) should be used.

TSH receptor antibody concentrations of the unknown samples are directly read off in IU/l (WHO 90/672) against the respective OD values.

Medizym® TRAb clone can be evaluated by Computer Assisted Analysis using software able to calculate curves with 4 parameter / sigmoid regression.

## TYPICAL EXAMPLE

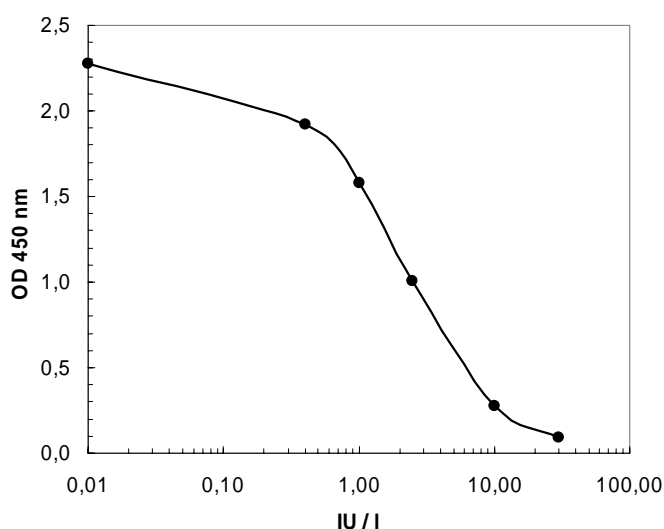
Do not use for evaluation!

well	OD (a)	OD (b)	OD (mean)	IU/l
Control I	2.238	2.310	2.274	<b>0.01</b>
Calibrator 1	1.967	1.872	1.920	<b>0.4</b>
Calibrator 2	1.563	1.595	1.579	<b>1.0</b>
Calibrator 3	1.011	1.002	1.006	<b>2.5</b>
Calibrator 4	0.277	0.272	0.274	<b>10</b>
Calibrator 5	0.095	0.084	0.089	<b>30</b>
Control II	0.979	1.058	1.018	<b>2.4</b>

The above mentioned standard concentrations and control values are only an example for a typical standard curve. They can change from lot to lot, see leaflet enclosed. Medizym® TRAb clone is strictly (1:1) calibrated against the WHO standard NIBSC 90/672.

## STANDARD CURVE

Typical example



### Criteria of validation

Specimens with an OD lower than the OD of calibrator 5 should be diluted by antibody-free serum and tested again. The results have to be multiplied with the dilution factor chosen.

## REFERENCE VALUES

Medizym® TRAb clone	IU/l
negative	≤ 0.3
grey zone	> 0.3 - 0.4
positive	> 0.4

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

## CHARACTERISTIC ASSAY DATA

### Linearity of Medizym® TRAb clone

Dilutions of specimens in TRAb-free human serum are determined according to their expected theoretical values with Medizym® TRAb clone. On the basis of the heterogeneous nature of the autoantibody population and in view of epitope specificity and affinity of the autoantibodies exceptions are possible in some cases.

### Specificity

Human TSH levels up to 3000 mIU/l did not show any significant cross reactivity in Medizym® TRAb clone.

### Sensitivity

The lower detection limit or **analytical sensitivity** (0 ± 3 S.D.) can regularly be determined at **0.1 IU/l**.

The most appropriate and statistically reasonable characteristic of any assay is at present the so-called **functional assay sensitivity**. This 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 Medizym® TRAb clone, this value is found at **0.3 IU/l**.

Medizym® TRAb clone values below this defined level of functional assay sensitivity do not meet the statistical criteria for reliability according to GLP (Good Laboratory Practice) and therefore can not be distinguished from zero due to the statistically necessary certainty.

Medizym® TRAb clone concentrations above 0.3 IU/l, however, fulfill these criteria and are consequently assessed as valid.

### Intra - and inter-assay variation

Intra-assay (n = 12)			Inter-assay (n = 5)		
Sample no.	Mean Concentration (IU/l)	CV (%)	Sample no.	Mean Concentration (IU/l)	CV (%)
1	0.26	16	5	0.3	14
2	0.8	6	6	0.7	12
3	6.8	4	7	1.8	8
4	20.0	3	8	6.0	6

At a cut-off value of 0.4 IU/l the sensitivity was found to be 97 % and the specificity is 100 % for patients with untreated Graves' disease.

## LIMITATIONS of the METHOD

Healthy individuals should be tested negative by the Medizym® TRAb clone.

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<sup>®</sup> TRAb clone

## ASSAY SCHEME

Bring all reagents to room temperature. Gently mix all reagents to ensure homogeneity.

Step	Activity	Material	CI, CAL 1 - 5	CII	Patients 1, 2, ...
1	Pipette	Incubation buffer (H)	75 µl	75 µl	75 µl
2	Pipette	Samples	75 µl	75 µl	75 µl
3	Incubate	Plate	120 minutes (20 - 25 °C) while shaking vigorously >500 rpm		
4	Aspirate or decant	Microtiter plate	put sharply onto absorbent tissue		
	Pipette	Washing solution (made from B)	1 x 300 µl	1 x 300 µl	1 x 300 µl
5	Pipette	M22 biotin complex	100 µl	100 µl	100 µl
6	Incubate	Plate	25 minutes (20 - 25 °C) without shaking *		
7	Aspirate or decant	Microtiter plate	put sharply onto absorbent tissue		
	Pipette	Washing solution (made from B)	1 x 300 µl	1 x 300 µl	1 x 300 µl
8	Pipette	Conjugate (made from D and J)	100 µl	100 µl	100 µl
9	Incubate	Plate	20 minutes (20 - 25 °C) without shaking *		
10	Aspirate or decant	Microtiter plate	put sharply onto absorbent tissue		
	Pipette	Washing solution (made from B)	3 x 300 µl	3 x 300 µl	3 x 300 µl
11	Pipette	Substrate (E)	100 µl	100 µl	100 µl
12	Incubate	Plate	30 minutes (20 - 25 °C) in the dark *		
13	Pipette	Stop solution (F)	50 µl	50 µl	50 µl
14	Measure OD	Plate	at 450 nm versus 620 (690) nm within 20 min		

- \* After adding reagents and before any incubation shake the plate for 5 seconds
- Only clear sera should be assayed (centrifuge, if necessary)

## 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 sodium azide and of Kathon GC 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:
  - D**
  - 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.