Instructions For Use

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Medizym®
T.R.A. human

REF 3505

Enzyme immunoassay for the determination of antibodies against TSH receptor in human serum

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IVD CE Σ 96 2°C 8°C

1 Intended Purpose

The Medizym® T.R.A. human is a quantitative immunoassay for the determination of antibodies against Thyrotropin (TSH) receptor in human serum.

The Medizym® T.R.A. human is intended as an aid in the diagnosis of Graves' disease in conjunction with other clinical and laboratory findings.

The immunoassay is designed for manual professional *in vitro* diagnostic use.

2 Diagnostic Relevance

In contrast to thyrotoxicosis which 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 T4 and T3. For this reason the measurement of these antibodies is valuable for the differential diagnosis of hyperthyroidism as well as for the follow-up of Graves' disease.

3 Test Principle

The competitive ELISA (Enzyme Linked Immunosorbent Assay) is an immunoassay for the determination of specific antibodies. The strips of the microtiter plate are coated with test-specific antigens. If antibodies are present in the patient´s sample, they bind to the antigens. A second biotinylated tracer antibody targets the remaining accessible binding sites of the coated antigen. Streptavidin conjugated with the enzyme peroxidase binds the immobilized biotinylated antibody. A colorless substrate is converted into the colored product by the peroxidase. The signal intensity of the reaction product is indirectly proportional to the antibody activity in the sample. After stopping the signal intensity of the reaction product is measured photometrically.

4 Test Components

Component	Description
Microtiter plate **A** **MP** 1 piece	12 breakable microtiter strips (ready-to-use), 8 wells per strip, each well coated with recombinant human TSH receptor
Calibrator **1 – 5** **CAL** 5 x 1.0 mL	Colored dilutions of human serum (ready-to-use; contains sodium azide) The antibody activities are indicated on the quality control certificate.
Positive control **P** **CONTROL | +** 1 x 1.0 mL	Colored dilution of human serum (ready-to-use; contains sodium azide) The antibody activity is indicated on the quality control certificate.
Incubation buffer **H** **START** 1 x 15 mL	Solution (ready-to-use; contains sodium azide and ProClin 950)
TSH-R antibody biotin **G** **TRA-Biotin** 1 x 15 mL	Dilution of biotinylated TSH receptor antibody (ready-to-use; contains ProClin 950)
Streptavidin-per-oxidase (SA-POD) **D** **CONJ** 1 x 0.75 mL	Concentrated streptavidin conjugated to horseradish peroxidase (20x)
Diluent for SA-POD **J** **BUF D** 1 x 15 mL	Solution (ready-to-use; contains ProClin 950)
Wash buffer **B** **WASHB** 1 x 100 mL	Concentrated solution (10x; contains ProClin 950)
Substrate **E** **SUB** 1 x 15 mL	3,3´,5,5´-Tetramethylbenzidine (ready-to-use)
Stop solution **F** **STOP** 1 x 15 mL	0.25 M Sulfuric acid (ready-to-use)
Adhesive Foil 2 pieces	-
QC Certificate 1 piece	-
Instructions for Use 1 piece	-

5 Materials required but not provided

- Common laboratory equipment
- Precision pipettes (5 – 1000 µL), multi-channel pipettes (100 – 1000 µL) and disposable pipette tips
- Graduated cylinders (100 – 1000 mL)
- Vortex mixer or other rotators
- Microtiter plate shaker
- Microtiter plate washer or wash comb
- Microtiter plate reader with optical filters for 450 nm and 620 nm or 690 nm
- Adsorbent paper or paper towel

- Distilled or de-ionized water

6 Storage and Stability

Upon receipt, all test components must be stored at 2 °C to 8 °C, preferably in the original kit box. If stored properly in their original containers, all components are stable until their expiry date.

7 General Information

This product is for *in vitro* diagnostic use only. The instructions for use must be carefully read before use. They are valid only for the present product with the given composition and must be strictly followed to ensure reliable test results. Deviations can lead to erroneous test results. Components must not be exchanged by test reagents of different lots or of other manufacturers.

Contamination of reagents must be avoided by use of aseptic techniques when removing aliquots from the vials. After use, reagent vials must be tightly closed with their corresponding caps. Cross-contamination of samples or reagents can lead to inconsistent test results and must be avoided by use of consistent pipetting techniques.

Exposure of reagents to strong light must be avoided throughout the entire test procedure and storage.

Insufficient washing will result in poor precision and elevated measurement signals. After each washing step any residual fluid has to be removed completely.

8 Preparation

8.1 Preparation of Reagents

All components including the microtiter plate must be brought to room temperature (RT: 18 °C to 25 °C) before use for at least 30 min. All liquid components must be mixed gently to ensure homogeneity.

8.1.1 Microtiter Plate

The microtiter plate is sealed in an aluminium bag. Unused test strips should always be stored refrigerated and protected from moisture with the desiccant in the properly sealed aluminum bag. Carefully resealed, the test strips can be used for 8 weeks after opening.

8.1.2 Calibrators

The calibrators are ready-to-use and must not be diluted any further. Calibrators must be used in each test run.

8.1.3 Controls

The positive control is ready to use and must not be diluted any further. Controls must be used in each test run. Laboratories can also validate their own control samples and use them alternatively.

8.1.4 Incubation Buffer

The incubation buffer is ready-to-use.

8.1.5 TSH-R antibody biotin

The solution of the biotinylated antibody against the TSH receptor is ready-to-use.

8.1.6 Streptavidin-peroxidase (D) and Diluent (J)

A sufficient amount of streptavidin-peroxidase solution is to be prepared by diluting SA-POD concentrate (D) 1 + 20 (e. g. 0,5 mL SA-POD concentrate with 10,0 mL diluent for SA-POD (J). The prepared SA-POD solution (D+J) must be used on the same day.

8.1.7 Wash Buffer

The wash buffer is concentrated and must be diluted 1:10 with distilled water before use (e. g. 100 mL + 900 mL). A sufficient amount of washing solution must be prepared. The diluted washing solution can be stored at 2 °C to 8 °C up to 30 days.

8.1.8 Substrate

The substrate is ready-to-use. Exposure of the substrate solution to strong light should be avoided.

8.1.9 Stop Solution

The stop solution is ready-to-use.

8.2 Preparation of Samples

8.2.1 Sample Material

The use of freshly collected serum from blood taken by venipuncture is recommended. The use of icteric, lipemic, hemolytic or bacterially contaminated samples should be avoided. Insoluble substances must be removed from the sample by centrifugation. Samples must not be thermally inactivated.

8.2.2 Sample Storage

Samples may be kept at 2 °C to 8 °C up to three days. Long-term storage requires -20 °C. Repeated freezing and thawing should be avoided. For multiple use, samples should be aliquoted and kept at -20 °C.

9 Test Performance

9.1 Pipetting Scheme

The following pipetting scheme is recommended:

	1	2	3	4
A	CAL 1	Sample 3		
B	CAL 2	Sample 4		
C	CAL 3	Sample 5		
D	CAL 4	…		
E	CAL 5	…		
F	P	…		
G	Sample 1	…		
H	Sample 2	…		

9.2 Procedure

The indicated incubation times and temperatures must be adhered to and significant time shifts during pipetting samples and reagents must be avoided. The microtiter plate should be shortly shaken after addition of reagents.

Step	Description
1. Addition of incubation buffer	Add 100 µL ready-to-use incubation buffer per well
2. Addition of calibrators, controls and samples	Add 100 µL ready-to-use calibrators, controls and undiluted samples per well and shake the plate shortly
3. Incubation	Cover the plate and incubate for 120 min. at RT while shaking at 500 rpm on a plate shaker
4. Wash cycle	Aspirate the solution and wash 3 times with 300 µL washing solution with at least 5 seconds soaking time each; dry by tapping the microtiter plate on a paper towel to remove any residual droplets
5. Addition of start reagent	Add 100 µL of TRA-biotin to each well
6. Incubation	Cover the plate and incubate for 20 min. at RT
7. Wash cycle	Aspirate the solution and wash 3 times with 300 µL washing solution with at least 5 seconds soaking time each; dry by tapping the microtiter plate on a

	paper towel to remove any residual droplets
8. Addition of conjugate	Add 100 µL of diluted SA-POD (prepared from D and J) to each well
9. Incubation	Cover the plate and incubate for 20 min. at RT
10. Wash cycle	Aspirate the solution and wash 3 times with 300 µL washing solution with at least 5 seconds soaking time each; dry by tapping the microtiter plate on a paper towel to remove any residual droplets
11. Addition of substrate	Add 100 µL ready-to-use substrate to each well
12. Incubation	Cover the plate and incubate for 20 min. in the dark at RT
13. Addition of Stop Solution	Add 50 µL ready-to-use stop solution to each well
14. Analysis	Read optical density (OD) at 450 nm versus 620 or 690 nm within 30 min. after stopping the reaction

9.3 Automation

Automated processing of the immunoassays must be performed analogous to manual use and validated by the user.

10 Test Evaluation

10.1 Metrological Traceability

The immunoassay is calibrated using the international WHO standard NIBSC code 08/204. Quantitative results are expressed in IU/L.

10.2 Quantitative Evaluation

For generation of a standard curve, the optical signals (optical density, OD) of the calibrators are plotted against their antibody activities and correlated by a 4-parameter logistic (4 PL) fit. Antibody activities of unknown samples can be derived directly from their optical signals by use of the generated standard curve.

10.3 Criteria of Validity

Test runs are only valid if the following criteria of validity are fulfilled:

- OD CAL 1 > CAL 2 > CAL 3 > CAL 4 > CAL 5
- OD CAL 1 > 1.2
- The positive control must be evaluated positive and present an antibody activity within the validity range indicated on the quality control certificate.

If these criteria are not met, the test is not valid and must be repeated.

10.4 Troubleshooting

In case of an invalid test run, the expiry dates and storage conditions, incubation times and temperatures, and precise calibration of all instruments used should be verified. If no reason for an invalid test run could be identified, please contact the supplier or manufacturer of the product.

10.5 Reference Ranges

The reference ranges are indicated below:

	Interpretation
Antibody activity < 1 IU/L	negative
Antibody activity 1.0 – 1.5 IU/L	borderline
Antibody activity > 1.5 IU/L	positive

As a result of different seroprevalences in individual regions, each laboratory should verify the reference ranges by own analysis and adapt, if necessary.

10.6 Interpretation of Test Results

A positive test result indicates the presence of specific antibodies. A negative result indicates the absence of specific antibodies, but does not exclude the possibility of an autoimmune reaction. In case of a borderline test result, a reliable evaluation is not possible.

10.7 Limitations of the Method

The interpretation of test results must always be considered in combination with the clinical picture of the patient. The diagnosis should not be based on the results of a sole diagnostic method. All clinical and laboratory findings should be evaluated to state a diagnosis. For confirmation, further investigations should be carried out.

11 Performance Characteristics

11.1 Analytical Performance Characteristics

11.1.1 Precision

The precision of test results was assessed by the determination of the intra- and interassay variation by the analysis of multiple samples with different antibody activities.

	Intraassay Precision		Interassay Precision	
	OD	CV (%)	OD	CV (%)
Sample 1	1.259	3.7	1.181	6.6
Sample 2	0.817	5.4	0.780	5.8
Sample 3	0.538	6.0	0.510	6.4

11.1.2 Measurement Range

Reliable accuracy, trueness, precision, linearity and recovery of test results have been observed within the measurement range of the assay from the LoQ to the upper calibrator in comprehensive studies. Samples with test results above the upper calibrator should be reported as >max. Samples with test results below the LoQ should be reported as <min. If test results above the upper calibrator are observed, the samples may be tested at a higher dilution. The resulting antibody activity must be multiplied with the additional dilution factor.

11.2 Diagnostic Performance Characteristics

11.2.1 Diagnostic Sensitivity and Specificity

Sensitivity and specificity were assessed by the analysis of serum samples from 48 patients with Graves' disease and 77 samples from unselected blood donors.

	Diagnostic Performance
Sensitivity	95 %
Specificity	99 %

12 Warnings and Precautions

The product is designed exclusively for *in vitro* diagnostic use by qualified, authorized and trained personnel. All test components and human samples should be handled with care as potentially hazardous. Good laboratory practices (GLP) and all relevant regulations should be adhered to.

In case the product is damaged or product information including labelling is wrong or incorrect, please contact the manufacturer or supplier.

This product contains preparations of human and / or animal origin. Any material derived from human body fluids or organs used for the preparation of components were tested and found negative for HBsAg (Hepatitis B-Virus-surface Antigen) and anti-HIV as well as anti-HCV antibodies. However, all components and all patient samples should be handled as potentially hazardous in accordance with national laws and appropriate guidelines on biological safety.

As the product contains potentially hazardous materials, the following precautions should be followed: Do not smoke, eat or drink while handling kit material or samples. Avoid direct contact to kit material or samples by wearing protective gloves laboratory coat and safety glasses. Never pipette material by mouth. Wipe up spills promptly and wash the affected surface thoroughly with a decontaminant. Wash hands thoroughly after use.

Some of the reagents contain ProClin (< 1.0 %) as a preservative, may cause skin sensitization (H317) and must not be swallowed or allowed to come into contact with skin or mucosa (P280, P333+P313).

Some of the reagents contain sodium azide (< 0.1 %) as a preservative and must not be swallowed or allowed to come into contact with skin or mucosa. The possible formation of heavy metal azides in the drainage has to be prevented by sufficient rinsing with water.

The information in the safety data sheet on possible hazards, first aid measures, measures in the event of the unintentional release of large quantities, handling and storage, personal protective equipment, information on disposal as well as information on toxicology must be observed.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the member state in which the user and/or the patient is established.

13 Disposal

For decontamination and disposal the recommendations of the CDC as well as the relevant local and national environmental guidelines and regulations should be adhered to. Samples, potentially contaminated materials and infectious waste must be decontaminated, e.g. by autoclaving for 20 min. at 121 °C.

14 References

- 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, 849 – 55.
- 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, 113 – 6.
- Roggenbuck JJ, Veiczi M, Conrad K, Schierack P, Wunderlich G, Kotzerke J, Roggenbuck D, Zöphel K. A novel third-generation TSH receptor antibody (TRAb) enzyme-linked immunosorbent assay based on a murine monoclonal TSH receptor-binding antibody. Immunol Res. 2018, 66, 768 – 76.

15 Symbols

	Manufacturer
CE	CE marking of conformity
IVD	*In vitro* diagnostic medical device
REF	Catalogue number
UDI	Unique device identifier
LOT	Batch code
	Temperature limit
	Use-by date
	Consult instructions for use
	Contains sufficient for <n> tests
	Do not re-use
	Caution
	Warning
	Biological risk
	Keep away from sunlight
MP	Microtiter plate
CAL	Calibrators
CONTROL	Control
TRA-Biotin	Biotin complex
START	Incubation buffer
BUF H	Diluent for start reagent
CONJ	Conjugate
BUF D	Diluent for conjugate
WASHB	Wash buffer
SUB	Substrate
STOP	Stop solution

16 Changes

Changes in current Instructions for Use	
Current Version	010/05.2025
Summary of Changes	Section 8.1.6: the ready-to-use SA-POD solution (D+J) must be used on same day