DiaSource® **Instructions For Use**

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TRAb human 1 step RIA

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Radioimmunoassay for the determination of antibodies against TSH receptor (TRAb) in human serum

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IVD CE Σ 100 2°C–8°C

1 Intended Purpose

The TRAb human 1 step RIA is a quantitative immunoassay for the determination of antibodies against Thyrotropin (TSH) receptor in human serum utilizing the coated tube technology.

The TRAb human 1 step RIA is intended as an aid in the diagnosis of autoimmune thyroid diseases in conjunction with other clinical and laboratory findings.

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

2 Diagnostic Relevance

The majority of TSH receptor autoantibodies (TRAb) mimic the action of TSH on thyroid cells and thus increase blood levels of T4 and T3. Due to absence of a negative feedback system, the stimulation of the thyroid gland often leads to the clinical thyrotoxic state of Graves' disease.

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, radioiodine therapy or surgery.

Pregnant women developing Graves' disease can transfer their autoantibodies to the fetus via the placental unit. The higher the TRAb concentrations of the mother, the greater the risk for the embryo to develop Graves' disease in uterus and thus to suffer from congenital hyperthyroidism.

3 Test Principle

Radioimmunoassays based on coated tube technology are widely used for the determination of specific antibodies. The TRAb human 1 step RIA is based on a competitive reaction of antibodies against the TSH receptor (TRAb) of the patient samples with a limited amount the iodinated monoclonal antibodies against the TSH receptor of the tracer for the immobilized TSH receptor on the solid phase of coated tubes. The antibody activity in the patient sample influences the tracer antibody activity binding to the TSH receptor on the surface of the tubes. Decantation of the reaction mixture allows for the simple separation of generated immune complexes on the surface of the tube from the reaction mixture. The radioactivity of the tube is measured and inversely proportional to the TSH receptor antibody activity in the sample.

4 Test Components

Component	Description
Coated Tubes **A** **CT** 2 x 50 pieces	Tubes coated with human TSH receptor (ready-to-use)
Calibrator **0 – 4** **CAL** 5 x 0.7 mL	Dilutions of human serum (ready-to-use; contain sodium azide) The antibody activities are indicated on the quality control certificate.
Low Control **CI** **CONTROL** 1 x 0.7 mL	Dilution of human serum (ready-to-use; contains sodium azide) The antibody activities are indicated on the quality control certificate.
High Control **CII** **CONTROL** 1 x 0.7 mL	Dilution of human serum (ready-to-use; contains sodium azide) The antibody activities are indicated on the quality control certificate.
Tracer **D** **TRAC** 1 x 12 mL	Monoclonal antibody against TSH receptor conjugated to Iodine-125 (< 0.39 MBq, t½ = 59 days; gamma radiation 35 keV, x radiation 27 keV, 31 keV; ready-to-use; contains sodium azide)
Start Buffer **H** **START** 1 x 10 mL	Solution (ready-to-use; contains ProClin 950 and sodium azide)
QC Certificate 1 piece	-
Instructions for Use 1 piece	-

5 Materials required but not provided

- Common laboratory equipment
- Precision pipettes (5 – 1000 µL) and disposable tips
- Graduated cylinders (100 – 1000 mL)
- Sample tube rack
- Plastic foil
- Vortex mixer or other rotators
- Shaker
- γ-counter
- Adsorbent or blotting paper
- 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.

8 Preparation

8.1 Preparation of Reagents

All components including 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. Foaming should be avoided.

8.1.1 Coated tubes

The coated tubes are sealed in a foil bag. Unused tubes should always be stored refrigerated and protected from moisture and have to be used up within 2 weeks.

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 controls are 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 Start Buffer

The start buffer is ready-to-use

8.1.5 Tracer

The tracer is ready-to-use and must not be diluted any further.

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 Procedure

A sufficient amount of tubes must be prepared. The indicated incubation times and temperatures must be adhered to and significant time shifts during pipetting samples and reagents must be avoided. It is recommended to add the tracer in blocks after every 25 tubes.

Step	Description
1. Addition of start buffer	Add 50 µL ready-to-use start buffer per test tube.
2. Addition of calibrators, controls, undiluted samples as well as tracer	Add 100 µL ready-to-use calibrators, controls and undiluted samples per test tube and add. 100 µL tracer material per test tube. Calibrators, controls and samples have to be mixed directly with tracer prior to dispensing. Seal all tubes with a plastic foil.
3. Incubation	Incubate the covered tubes for 2 hours at RT on a shaker. Adjust the speed to allow constant rotating of the liquid (> 250 rpm).
4. Addition of distilled water	Aspirate the solution and wash 2 times with 1.0 mL of distilled water to each tube
5. Decantation	Decant the supernatant from all tubes by inversion of the rack. For removal of any remaining liquid, turn tubes upside down (5 min.) and absorb any droplets by tapping on blotting paper.
6. Analysis	Measure radioactivity of all tubes including reference T with a γ-counter and a recommended counting time of 1 min.

9.2 Automation

Automated processing of the immunoassays can be performed with RIAmat SR280/SR300 analogous to manual use and validated by the user.

10 Test Evaluation

10.1 Metrological Traceability

The immunoassay is calibrated using the international WHO reference preparation 08/204 (2nd International Standard for Thyroid Stimulating Antibody). Quantitative results are expressed in IU/L.

10.2 Quantitative Evaluation

The evaluation is based on the measured radioactivity (counts per minute; cpm) of all calibrators, controls and patient samples. An evaluation based on binding rates B/B_0 (%) is also possible. Binding rates B/B_0 (%) are calculated by division of the measured radioactivity (counts per minute; cpm) of all calibrators, controls and patient samples with the measured radioactivity (counts per minute; cpm) of CAL 0.

For generation of a standard curve, the measured radioactivity (cpm) or the calculated binding rates B/B_0 (%) of the calibrators are plotted against their concentrations and correlated by spline smoothing. Concentrations of unknown samples can be derived directly from their measured radioactivity (cpm) or calculated binding rates B/B_0 (%) 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:

- cpm or B/B_0 (%) CAL 4 < CAL 3 < CAL 2 < CAL 1 < CAL 0
- The controls must present an antibody concentrations 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 < 1.0 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

Values out of the reference range indicate disorder of thyroid metabolism. Values below the reference range can be associated with hypothyroidism, values above the reference range should be considered in the context of hyperthyroidism. Values of free thyroxine (FT4) and thyroidal stimulating hormone (TSH) must additionally be included in the interpretation.

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 Analytical Sensitivity and Specificity

The Limit of Blank (LoB) was determined by multiple analysis of sample diluent. The Limit of Detection (LoD) and the Limit of Quantitation (LoQ) were assessed by multiple analysis of negative samples.

	Analytical Sensitivity
Limit of Blank (LoB)	0.02 IU/L
Limit of Detection (LoD)	0.17 IU/L
Limit of Quantitation (LoQ)	0.90 IU/L

The analytical specificity was assessed by addition of potentially interfering substances to samples and determination of their influence on the measurement. A significant influence of bilirubin (up to 0.15 mg/mL), hemoglobin (up to 10 mg/mL), triglycerides (up to 5 mg/mL) and EDTA (up to 2.5 mg/mL) on test results was not observed.

11.1.2 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 antigen concentrations.

	Intraassay Precision		Interassay Precision	
	IU/L	CV (%)	IU//L	CV (%)
Sample 1	16.5	5.2	14.6	7.7
Sample 2	3.3	7.4	2.8	7.9
Sample 3	1.1	10.3	0.8	15.4

11.1.3 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 concentration 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 101 serum samples from patients with Graves´ disease and 64 serum samples from unselected blood donors in comparison to the results of a competitor´s assay for the detection of TSH receptor antibodies.

	Diagnostic Performance
Sensitivity	98 %
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 and / or radioactive 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.

Some of the reagents contain radioactive material. Contaminated tissues, tubes, bench covers, gloves etc. must be disposed in a specially marked container. Discard liquid and solid radioactive waste only as permitted by federal, state or local authorities and regulations. It is the responsibility of the user of this product to handle radioactive material in accordance to the national rules given by law or other statements of the local authorities.

14 References

- Rees Smith B, McLachlan SM, Furmaniak J.: Autoantibodies to the thyrotropin receptor. Endocr. Rev. 1988, 9, 106 – 21.
- Rapoport B, Chazenbalk GD, Jaume JC, McLachlan SM.: The thyrotropin (TSH) receptor: interaction with TSH and autoantibodies. Endocr. Rev. 1998, 19, 673 – 716.
- Kakinuma A, Morimoto I, Kuroda T, Fujihira T, Eto S, McLachlan SM, 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.
- Zimmermann-Belsing T, Nygaard B, Rasmussen AK, Feldt-Rasmussen U.: Use of the 2nd generation TRAK human assay did not improve prediction of relapse after antithyroid medical therapy of Graves´disease; Eur. J. Endocrinol. 2002, 146, 173 – 7.
- 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. Endocr J. 2003, 50, 113 – 6.
- Roggenbuck JJ, Zarske G, Schierack P, Wunderlich G, Conrad K, Kotzerke J, Roggenbuck D, Zöphel K: Third generation radioimmunoassay (RIA) for TSH receptor autoantibodies (TRAb) - one step less, similar results? Nuklearmedizin 2021, 60, 38 – 46.

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
Σ	Contains sufficient for <n> tests
	Do not re-use
	Caution
	Biological risk
	Warning
	Keep away from sunlight
	Radioactive material or ionizing radiation
CT	Coated tube
CAL	Calibrator
CONTROL	Control
START	Start reagent
TRAC	Tracer

16 Changes

Changes in current Instructions for Use	
Current Version	004/08.2023
Summary of Changes	Actualization in Chapter 12