EliA™

Not for use in the USA

anti-TSH-R

FLUOROENZYMEIMMUNOASSAY FOR TSH-RECEPTOR ANTIBODIES

FOR IN VITRO DIAGNOSTIC USE

DIRECTIONS FOR USE

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EliA uses a modular reagent system. All information needed to understand the use of the EliA tests can be found in the analyte specific DfU and the corresponding EliA Control DfU.

INTENDED USE

EliA anti-TSH-R is intended for the in vitro quantitative measurement of autoantibodies to the thyroid stimulating hormone receptor (TSH-R) in human serum using a thyroid stimulating monoclonal antibody, as an aid in the clinical diagnosis of Graves' disease (autoimmune hyperthyroidism). EliA anti-TSH-R uses the EliA anti-TSH-R method on the instrument Phadia 250.

SUMMARY AND EXPLANATION OF THE TEST

Thyroid autoimmunity comprises a number of distinct but pathogenically related autoimmune disorders of the thyroid gland, such as Graves' disease or Hashimoto's thyroiditis. Among other things, these diseases are characterized by the presence of autoantibodies that are directed against thyroid antigens, such as TSH receptor, thyroglobulin (TG) or thyroid peroxidase (TPO, formerly known as the thyroid microsomal antigen).[1]

Graves' disease is a common autoimmune disorder with the most common manifestation being hyperthyroidism caused by antibodies binding to and activating the TSH-receptor on the follicular cells of the thyroid. Therefore, the vast majority of patients newly diagnosed with Graves' disease have detectable anti-TSH-R antibodies in the serum. Antithyroid drug (ATD) therapy of Graves' hyperthyroidism is in many patients followed by a remission of the autoimmunity of Graves' disease with a gradual disappearance of anti-TSH-R antibodies from the circulation[1,2,3].

The TSH receptor in the EliA anti-TSH-R well is a human recombinant protein which is also used in the well established high quality BRAHMS TRAK human tests from Thermo Fisher Scientific.

PRINCIPLES OF THE PROCEDURE

EliA anti-TSH-R is a competitive enzyme immunoassay. The EliA anti-TSH-R Wells are coated with human recombinant TSH-R and can be used for measuring of patients' samples as well as calibrators and curve controls.

Anti-TSH-R antibodies in patients' samples, calibrators and controls bind to the coated human TSH-R. After washing away non-bound components, enzyme-labeled recombinant antibodies (EliA anti-TSH-R Conjugate) are added to form a TSH-R-conjugate complex, if the TSH-R is not blocked by antibodies from patients' serum, calibrator or control.

After incubation, non-bound conjugate is washed away and the bound complex is incubated with Development Solution. After stopping the conjugate-enzyme reaction, the fluorescence activity, corresponding to the bound enzyme-labelled conjugate, is measured. The lower the response value of the fluorescence, the less conjugate is bound to coated TSH-R, because more antibodies from patients' samples, calibrator or control are bound to coated TSH-R.

To evaluate test results, the response for the patient samples is compared directly to the response of the calibrators.

REAGENTS / MATERIAL

EliA reagents are available as modular packages, each sold separately. All packages except for the EliA Thyroid Positive Control 250 and the EliA IgG/IgM/IgA Negative Control 250 are required to carry out an EliA anti-TSH-R test. The EliA anti-TSH-R Wells are packed in carriers which are stored in sealed aluminium foil bags containing a desiccant.

EliA anti-TSH-R Test-Specific Reagents

EliA anti-TSH-R Well (Art. No. 14-5639-01)

anti-TSH-R Well; short name: tsr	coated with human recombinant TSH receptor antigen	4 carriers (16 wells each); sufficient for 64 determinations	ready for use; store dry at 2-8 °C until expiration date

EliA Thyroid Positive Control 250 (Art. No 83-1113-01)

Human serum and monoclonal antibody in PBS containing BSA, detergent and sodium azide (0.095%); symbol: pos	Control containing IgG antibodies to TPO, TG and TSH-R	6 single-use vials (0.3 ml each); sufficient for 2 determinations per vial	Ready for use; store at 2-8 °C until expiration date

EliA Thyroid Positive Control 250 is prepared from selected pooled human sera and monoclonal antibody.

EliA IgG/IgM/IgA Negative Control 250 (Art. No 83-1037-01)

Human serum in PBS containing BSA, detergent and sodium azide (0.095 %); symbol: neg	Multiparameter control containing normal sera from healthy donors	6 single-use vials (0.3 ml each); sufficient for 2 determinations per vial	Ready for use; store at 2-8 °C until expiration date

EliA IgG/IgM/IgA Negative Control 250 is prepared from selected pooled human sera.

EliA Method-Specific Reagents (Phadia 250)

EliA anti-TSH-R Sample Diluent (Art. No 83-1121-01)

Sample Diluent (green colored); PBS containing BSA, EDTA, detergent and sodium azide (0.095 %)	6 bottles (48 ml each); sufficient for ≥6 x 800 dilutions	ready for use; store at 2-8 °C until expiration date

EliA anti-TSH-R Conjugate 50 (Art. No 83-1109-01)

anti-TSH-R Conjugate (blue colored); ß-Galactosidase anti-TSH-R (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: EI-T	6 wedge shaped bottles (5 ml each); sufficient for 6 x 50 determinations	ready for use; store at 2-8 °C until expiration date DO NOT FREEZE DO NOT REUSE

EliA anti-TSH-R Conjugate 200 (Art. No 83-1110-01)

anti-TSH-R Conjugate (blue colored); ß-Galactosidase anti-TSH-R (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: EI-T	6 wedge shaped bottles (19 ml each); sufficient for 6 x 200 determinations	ready for use; store at 2-8 °C until expiration date DO NOT FREEZE DO NOT REUSE

EliA anti-TSH-R Calibrator Strips (Art. No 83-1111-01)

mouse anti-TSH-R (0, 1.5, 3.5, 6.5, 12.5, 40 IU/l); in PBS containing BSA, detergent and sodium azide (0.095 %)	5 strips 6 single-use vials per strip (0.3 ml each); sufficient for one calibration curve (double determination)	ready for use; store at 2-8 °C until expiration date

EliA anti-TSH-R Curve Control Strips (Art. No 83-1112-01)

mouse anti-TSH-R (6.5 IU/l); in PBS containing BSA, detergent and sodium azide (0.095 %) symbol: CC-1	5 strips Each strip contains 6 x 0.3 ml CC-1 (double determination)	ready for use; store at 2-8 °C until expiration date

Phadia 250 General Reagents

Development Solution (Art. No. 10-9440-01)

Development Solution 0.01 % 4-Methylumbelliferyl-β-D-galacto-side, <0.0010 % preservative*	6 bottles (17 ml each); sufficient for 6 x >170 deter-minations	ready for use; store at 2-8 °C until expiration date DO NOT FREEZE

Development Solution (Art. No. 10-9441-01)

Development Solution 0.01 % 4-Methylumbelliferyl-β-D-galacto-side, <0.0010 % preservative*	6 bottles (11 ml each); sufficient for 6 x >110 deter-minations	ready for use; store at 2-8 °C until expiration date DO NOT FREEZE

Stop Solution (Art. No. 10-9442-01)

Stop Solution 4 % Sodium Carbonate	6 bottles (119 ml each); suffi-cient for 6 x >560 determinations	ready for use; store at 2-32 °C until expiration date

Stop Solution (Art. No. 10-9479-01)

Stop Solution 4 % Sodium Carbonate	6 bottles (65 ml each); sufficient for 6 x >292 determinations	ready for use; store at 2-32 °C until expiration date

Dilution Plates (Art. No. 12-3907-08)

MicroWell ™ plates with 96 wells, 0.5 ml each; Polypropylene	100 plates per package; sufficient for 100 x 96 samples	ready for use DO NOT REUSE

* Preservative: mixture of 5-chloro-2-methyl-2H-isothiazol-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1).

Washing Solution (Art. No. 10-9422-01/10-9202-01)

For information see separate Washing Solution package insert.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use reagents beyond their expiration dates.
- We do not recommend to pool reagents.
- Some of the reagents are manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, for antibodies to HIV1, HIV2 and hepatitis C virus and found negative. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed. Please refer to Human Health Service (HHS) Publication No. (CDC) 93-8395 or local and national guidelines on laboratory safety procedures.

WARNING! Reagents contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or absorbed by skin or eyes. NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local and national guidelines.
Waste Bottle and ImmunoCAP/EliA Well Waste Container may be contaminated by potentially infectious material. Use appropriate safety measures and wear gloves.

Indication of Instability

Phadia 250 Instrument Software has built-in acceptance limits for the calibration curve and the curve control. EliA Wells are moisture sensitive. An activity loss that might occur due to inappropriate handling can be detected using the appropriate EliA Control. For more information see Phadia 250 User's Guide/Reference Manual.

INSTRUMENT

The Phadia 250 Instrument processes all steps of the test. For further information regarding test set-up, instrumentation and software etc. see Phadia 250 User's Guide/ Reference Manual.

SPECIMEN COLLECTION, HANDLING AND PREPARATION

The procedure can be performed with serum specimens. Lipemic, hemolyzed or microbially contaminated samples may give poor results and should not be used.

- Undiluted samples should remain at room temperature for no longer than eight hours.*
- Undiluted samples can be stored at 2-8°C for two weeks without degradation provided they do not become contaminated by bacteria or fungi and they should be frozen at below -20°C for any long-term storage.**

Note: It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory. In general, laboratories should perform validation studies before implementing a change in specimen acceptance criteria.*

* Clinical and Laboratory Standards Institute (CLSI). Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline – Fourth Edition. CLSI document H18-A4 (ISBN 1-56238-724-3)
** Protein reference units-Handbook of Autoimmunity, 4th edition, A. Milford, Joanna Sheldon, G.D. Wild. Page 14.

Sample Dilution

Samples must be diluted with EliA anti-TSH-R Sample Diluent. A 1:2 dilution of the samples is required for the EliA anti-TSH-R test. Samples can be diluted manually, but instrument dilution is recommended.

PROCEDURE

Handling of EliA anti-TSH-R Well

In the Phadia 250 storage chamber, carriers are stable for up to 28 days. If you are not expecting to use them up within this time, the carriers should be loaded via the Phadia 250 Loading Tray and, for stability reasons, must be put back into the desiccant-containing foil bag directly after the run. Because it is important to store the wells in dry conditions at 2-8°C, the bag must be properly resealed. If stored under these conditions, the shelf-life from the date of first opening is 9 months, if not limited by the expiry date stated on the carrier and foil bag.

Lot specific barcode

Use the built-in barcode reader to enter the lot specific information of EliA anti-TSH-R Well and EliA anti-TSH-R Conjugate. In case of manual handling make sure to enter the characters below the barcode.

On-board stability of reagents

- **EliA Wells**
  EliA Well carriers can be stored on-board for 28 days at 2-8°C or 24 hours at room temperature.
- **EliA anti-TSH-R Calibrator Strips, EliA anti-TSH-R Curve Control Strips**
  Can be stored on-board for 28 days.
- **EliA anti-TSH-R Sample Diluent**
  Can be stored on-board for 7 days at room temperature. Re-cap bottles every night.
- **EliA anti-TSH-R Conjugate**
  Single use reagent, open vials must not be stored.
- **Development Solution**
  Can be stored on-board for a total of 40h at room temperature. Can be used 5 times during shelf life and be stored at room temperature for 8 hours on each occasion. Re-cap bottles every night. During weekends or longer interval between instrument usage it is recommended to store bottles at 2-8°C.

- **Stop Solution**
  Can be stored on-board for 7 days at room temperature. Re-cap bottles every night.
- **Washing Solution**
  Prepared solution can be stored on-board for 7 days at room temperature. Discard every seventh day and perform weekly maintenance according to instrument user manual.

Volumes per determination

Reagent volumes per determination

Calibrator	90 µl
EliA anti-TSH-R Conjugate	90 µl
Development Solution	90 µl
Stop Solution	200 µl

Sample volumes per determination

Manual dilution:	90 µl of diluted sample
Instrument dilution (1:2):	45 µl of non diluted sample

For tube-specific dead volumes see Phadia 250 User's Guide/Reference Manual.

Reagent volumes per 200 determinations

Washing Solution	5-7 l*
Rinse Solution	5-6 l*

* The residual volume depends on the number of samples and dilution method used.

Procedural comments

- When using software default, samples are run in single determination.
- Washing Solution must be at room temperature when used.
- The first result is available after approx. 2 hours and further results at one minute intervals afterwards. Up to 5 x 10 samples can be loaded continuously and are processed by random access.
- Incubations are automatically performed at 37 °C (98.6 °F).

CALIBRATION AND REFERENCE MATERIAL

The calibration curve is obtained with EliA anti-TSH-R Calibrators which are run in duplicate. The curve is stored and subsequent tests are evaluated against the stored curve using only the EliA anti-TSH-R Curve Control (run in duplicate).
The EliA anti-TSH-R Calibrators are traceable via an unbroken chain of calibrations to the WHO International Standard 2nd International Standard for Thyroid Stimulating Antibody NIBSC code: 08/204.[7]

A new calibration curve must be run when:

- the last calibration was made more than one month ago or
- a new lot of EliA anti-TSH-R Conjugate or a new EliA anti-TSH-R well lot is introduced or
- when the EliA anti-TSH-R Curve Control is outside the specified limits (defined in Phadia 250 Instrument Software).

EliA anti-TSH-R is calibrated against the WHO International Standard 2nd International Standard for Thyroid Stimulating Antibody NIBSC code: 08/204. Results are given in International Units (IU/l).

QUALITY CONTROL

Control Specimens

Good laboratory practice requires that quality control specimens should be included in every run. Any material used should be assayed repeatedly to establish mean values and acceptance ranges. EliA Controls are available for the quality control of the measurements.

CALCULATION AND INTERPRETATION OF RESULTS

Presentation of Results

Phadia 250 measures specific anti-TSH-R antibody concentrations in arbitrary U/l. By using a conversion factor given by the lot-specific code of the EliA anti-TSH-R Well, the results are automatically converted to IU/l. In contrast to most other anti-TSH-R assays on the market, the international units are based on the WHO International Standard 2nd International Standard for Thyroid Stimulating Antibody (NIBSC code: 08/204) and not on the first (NIBSC code: NIBSC 90/672).[7] Therefore, the IU/l are not directly comparable to the units of other anti-TSH-R assays.

Interpretation of Test Results

The ranges (negative, equivocal, positive) recommended for the evaluation of the results are given in the table below.

Test	Unit	negative	equivocal	positive
EliA anti-TSH-R	IU/l	< 2.9	2.9 – 3.3	> 3.3

Good laboratory practice requires that each laboratory establishes its own range of expected values.

LIMITATIONS

A definitive clinical diagnosis should not be based on the results of a single diagnostic method, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

EXPECTED VALUES

Antibody prevalence in autoimmune patients varies widely depending on disease area. However, second-generation assays using recombinant human TSH-receptor are said to be highly specific[4]. Antibodies to TSH-R have been reported in the lower single digit percentage of sera from healthy individuals[5,6,8].
Expected values may vary depending on the population tested.

Results Obtained for Healthy Subjects

The frequency distribution for anti-TSH-R antibodies was investigated in a group of apparently healthy subjects equally distributed by age and gender, using sera from a Caucasian population obtained from a blood bank. The results are given in the table below.

Test	Unit	No. of samples	Median value	95%-percentile	98%-percentile
EliA anti-TSH-R	IU/l	400	1.5	2.6	2.7

PERFORMANCE CHARACTERISTICS

Measuring Range

The measuring range (detection limit, upper limit) for EliA anti-TSH-R is from 1.5 to 80 IU/l. No hook effects could be observed for concentrations up to 20 fold above the measuring ranges.

Only values above the Detection Limit can be regarded as valid results. Results above the upper limit are reported as “> 80”.

Please note that due to differing binding characteristics of the antibodies in patient samples, not all sera can be diluted linearly within the measuring range.

Specificity

The EliA anti-TSH-R test permits the determination of TSH-R antibodies directed against the TSH-R antigen as described in section “Reagents”.

Precision

To determine the precision of the assay, the variability was assessed in a study with 21 runs by examining the samples in 252 replicates on 3 instruments over 7 days with a calibration curve included into each run.

Test	Sample	Unit	Mean value	Coefficients of variation (%)	
				Intra-Run	Inter-Run
EliA anti-TSH-R	1	IU/l	3.2	10.6	11.4
	2	IU/l	25.5	3.4	3.2
	3	IU/l	53.0	4.2	3.8

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Phadia AB may affect the results, in which event Phadia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Phadia AB and its authorized distributors, in such event, shall not be liable for damages, indirect or consequential.

REFERENCES

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PATENTS

European Patents 1021721 B1, EP 1456234 B1 and EP 1 565 493 B1, US Patents 8,309,693 B2, 8,298,769 B2, 8,753,637 B2 and 8,900,823 B2, Chinese patents CN1717418B and 101799476 B, Indian patents 226719 and 219312 apply.

LOT	Batch code	Σ	Contains x determinations
	Biological Risk		Read Directions for Use
	Store at 2-8°C/35-46°F		Manufactured by
	Expiration date	2	Do not reuse in a second run
IVD	For *in vitro* diagnostic use		

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