Instruction For Use

【Product Name】

Anti-TSHR (eCLIA)

【Order Information】

REF NO.	Package Size
0320511301	50T
0320511302	2×50T
0320511303	100T

【Intended Use】

Immunoassay for the in vitro quantitative determination of thyroid stimulating hormone receptor antibody (Anti-TSHR) in human serum. Determination of Anti-TSHR is used for auxiliary diagnosis of Graves' disease.

【Summary】

Anti-TSHR is an autoantibody in the serum of patients with autoimmune thyroid diseases, which can bind to thyroid stimulating hormone receptor (TSHR) to produce biological effects similar to TSH. Since it is not controlled by the negative feedback system, irritating thyroid action often leads to clinical hyperthyroidism symptoms of Graves' disease [1-3].

【Test Principle】

Competition principle. Total duration of assay: 18 minutes.

Anti-TSHR in the sample (50 µL) and Anti-TSHR labeled with ruthenium complex and competitively bind to the biotinylated thyroid stimulating hormone receptor (TSHR) forming an antibody-antigen complex, which is bound to streptavidin-coated microparticles, and then aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with system reagent. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent RIFD.

【Main Components】

The reagent pack consists of MB, RA, RB empty bottle (for PTR redissolved with PTB), calibrators and control materials (optional). Different lots cannot be used at the same time or mixed up together.

Components	Ingredients	Volume (2×50T)	Volume (50T)	Volume (100T)
Magnetic Beads (MB)	Streptavidin-coated microparticles, 0.45 mg/mL; ProClin300	2×1.8 mL	1×1.8 mL	1×3.5 mL
Reagent A (RA)	TSHR antibody labeled with ruthenium, 0.2 mg/L; PBS; ProClin300	2×3.0 mL	1×3.0 mL	1×6.0 mL
Reagent B (RB)	Empty bottle	2 PCS	1 PCS	1 PCS
Pretreatment reagent (PTR)	Biotinylated TSHR, 0.1 mg/L; MES; ProClin300; lyophilized	2×4.0 mL	1×4.0 mL	2×4.0 mL
Pretreatment buffer (PTB)	MES; ProClin300	2×5.0 mL	1×5.0 mL	2×5.0 mL
Calibrators	TSHR antibody; PBS; ProClin300	High: 1×1.0 mL Low: 1×1.0 mL		
Control Materials (Optional)	TSHR antibody; PBS; ProClin300	High: 1×2.0 mL Low: 1×2.0 mL		

Traceability: This method has been standardized against the 2nd international standard for Thyroid Stimulating Antibody (NIBSC, 08/204).

See the control material value sheet for the target values and ranges of control materials.

Supporting instruments and materials required but not provided in this pack (provided by Lifotronic)

Additional materials for Automated ECL Immunoassay Analyzer eCL8000, eCL8000i, eCL8000p, eCL8000x:

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzer eCL9000, eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 0320510002, Assay Cup/Assay Tip, 1×6×105

【Storage and Shelf Life】

Store at 2ºC-8°C.

Do not freeze.

Store the reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Damaged, expired or contaminated reagents should be discarded.

Stability of the reagents	
Unopened at 2~8°C	18 months
Store at 2~8°C after opening	4 weeks
Stored on the analyzers at 2~15°C	3 weeks

The calibrators and control materials are stable up to the stated expiration date.

Stability of the calibrators and control materials	
Unopened at 2~8°C	18 months
Store at 2~8°C after opening	4 weeks

Store calibrators and control materials upright in order to prevent the calibrator solution and control material solution from adhering to the cap of the vial.

Stability of the pretreatment reagent (PTR)	
Unopened at 2~8°C	18 months
Store at 2~8°C after reconstitution (RB)	4 weeks
Stored on the analyzers at 2~15°C (RB)	3 weeks

Pre-use treatment of the lyophilized pretreatment reagent (PTR):

50T, 2×50T: 4.0 mL pretreatment buffer (PTB) was added to each PTR bottle accurately by transferpettor for redissolving, respectively, and the fully dissolved and mixed PTR solution was transferred to the RB empty bottle. Avoid foam formation. Do not pour all PTB into PTR for reconstitution directly.

100T: 4.0 mL pretreatment buffer (PTB) was added to the 2 bottles of PTR accurately by transferpettor for redissolving, respectively, and all the 2 bottles of PTR solution after being fully dissolved and mixed were transferred to the RB empty bottle. Avoid foam formation. Do not pour all PTB into PTR for reconstitution directly.

【Applicable Instrument】

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【Specimen collection, handling and storage】

It is recommended to use human serum samples.

Blood samples should be collected in accordance with the standard operation of venipuncture. After the sample is completely coagulated, centrifugation should be performed to remove residual cellular substances. The sample should be free of air bubbles during testing. Lipid layer covering the sample after centrifugation should be removed. It is not recommended to use hemolyzed samples.

Samples can be stored for 7 hours at 18~28ºC, 6 days at 2~8ºC, and 12 months at -15ºC or below. Freezing and thawing can be performed once.

【Assay Procedure】

Testing procedures and precautions

Previous to operation, users should read the operational manual carefully to obtain system operation procedure, sample processing, security precaution, maintenance and other related information.

Set up Anti-TSHR test according to the operational manual.

Put the Anti-TSHR reagent pack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.

Recommended ambient temperature: 10ºC~30ºC; Relative humidity: ≤ 80%.

Calibration

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent and main curve information should be imported into the analyzer via radio frequency identification reagent card (refer to instrument user manual). The analyzer adjusts the main curve to produce working curve according to the calibrator results, and identifies validity of the working curve automatically.

Recalibration is recommended when:

(1) The reagents of different lot are used;

(2) The same lot of reagents were used on the analyzer beyond 21 days;

(3) Quality control findings outside the defined limits.

(4) The Buffer of different lots are used.

Quality control

For quality control, use Anti-TSHR Control Materials.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least

once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Please make sure that the correct values are used.

Calculation

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is IU/L.

The sample dilution

Samples with Anti-TSHR concentrations above the test range can be diluted with sample diluent. The recommended dilution is 1:10 (automatic dilution by instrument or manual dilution). If manually diluted, the result should be multiplied by the dilution. If the instrument is automatic dilution, the instrument will automatically calculate the result.

【Biological Reference Intervals】

Using the Anti-TSHR (eCLIA) assay on samples from 145 healthy individuals, 123 patients with thyroid diseases without diagnosis of Graves' disease, and 121 patients with untreated Graves' disease an optimal cutoff of 1.752 IU/L was determined. At this cutoff the sensitivity was calculated at 97.52% and the specificity at 99.25%. The calculated receiver operating characteristic (ROC) curve had an area under the curve (AUC) of 0.9991. The upper limits of Anti-TSHR values in the cohorts of healthy individuals and patients with thyroid disease without diagnosis of Graves' disease were 1.223 IU/L and 1.581 IU/L, respectively (95th percentiles).

Due to the differences in geography, race, gender and age, it is recommended that each laboratory determine the applicability of reference range through tests, and establish the reference range of this laboratory if necessary.

【Interpretation of results】

When interpreting the test results, it is necessary to refer to the patient's overall clinical situation, including: symptoms, medical history as well as other corresponding data and information.

【Limitations】

The test results are only for clinical reference and cannot be used alone as the basis for diagnosis or exclusion of cases.

The detection range of the kit is 0.5~40 IU/L. Values below the lower detection limit are reported as <0.5 IU/L; Values above the upper detection limit are reported as > 40 IU/L (10-fold diluted sample is reported as > 400 IU/L).

When the sample containing bilirubin ≤ 25 mg/dL, hemoglobin ≤ 400 mg/dL, triglyceride ≤ 1500 mg/dL, biotin ≤ 20 ng/mL, the interference deviation of the measured results lies within ±15%. When the rheumatoid factor concentration reached 600 IU/mL, the relative recovery of the test results is between 85%~115%.

Samples containing Anti-TG (4000 IU/mL), Anti-TPO (600 IU/mL), LH (10000 mIU/mL), HCG (50000 mIU/mL), FSH (10000 mIU/mL) were tested, and the determination results were not higher than 1.752 IU/L.

【Analytical Performance】

Lower Limits of Measurement

Limit of Blank = 0.5 IU/L.

Limit of Detection = 0.8 IU/L.

Limit of quantitation = 1.1 IU/L.

Accuracy

The relative deviation shall not exceed ±15%.

Linearity

The correlation coefficient is not less than 0.9900 in the interval of 0.8 ~ 40 IU/L.

Within-run precision (repeatability)

The coefficient of variation (CV)≤ 8.0%.

Between-lot precision

The coefficient of variation (CV) ≤10.0%.

Homogeneity of calibrators and control materials

Within-bottle homogeneity:

Calibrator (low): The standard deviation (SD) ≤ 0.3 IU/L.

Calibrator (high) and control materials: The coefficient of variation (CV) ≤ 8.0%.

Accuracy of calibrators

The relative deviation shall not exceed ±15%.

Measured values of control materials

The measured results of supporting assigned control materials of the kit should be within the quality control range.

Method comparison

A comparison of the Lifotronic Anti-TSHR assay (y) with the Roche Elecsys Anti-TSHR Method (x) using clinical samples gave the following correlations:

Number of serum samples measured: 111

Passing-Bablok regression: Linear regression:

T = 0.999 r=0.9975

The sample concentrations were between 0.806 and 36.31 IU/L.

【Precaution and Warning】

The kit is only used for in vitro diagnostics.

Exercise the normal precautions required for handling all laboratory reagents.

All reagents and samples including specimen, reagents, calibrators and control materials should avoid foaming before and during test.

Safety data sheet available for professional user on request.

This product contains animal-derived substances, and may have potential biological risks. All samples and reaction wastes should be treated as the source of infection, and all wastes must be disposed of in accordance with local regulations.

Any serious incident that would be 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.

The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1): May cause an allergic skin reaction. thus, proper personal protection should be adopt while handling the reagent: avoid breathing dust/fume/gas/mist/vapours/spray, wear protective gloves and contaminated work clothing should not be allowed out of the workplace. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and dispose of contents/container to an approved waste disposal plant.

【Literature References】

1. Orgiazzi J. Anti-TSH receptor antibodies in clinical practice. Endocrinol Metab Clin North Am. 2000 Jun;29(2):339-55, vii. doi: 10.1016/s0889-8529(05)70135-3. PMID: 10874533.
2. McIntosh RS, Asghar MS, Weetman AP. The antibody response in human autoimmune thyroid disease. Clin Sci (Lond). 1997 Jun;92(6):529-41. doi: 10.1042/cs0920529. PMID: 9205412.
3. Costagliola S, Morgenthaler NG, Hoermann R, et al. Second generation assay for thyrotropin receptor antibodies has superior diagnostic sensitivity for Graves' disease. J Clin Endocrinol Metab. 1999 Jan;84(1):90-7. doi: 10.1210/jcem.84.1.5415. PMID: 9920067.

【Symbols Explanation】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use-by date	IVD	In vitro diagnostic medical device
LOT	Batch code		Sufficient for <n> tests
SN	Serial number		Biological risks
	Temperature limit		This way up
EC REP	Authorized representative in the European Community	CE	Indicates this device is in compliance with Europe Directive.
REF	Catalogue number	P/N	Material code
	Warning		Date of manufacture
UDI	Unique device identifier		

【Manufacturer】

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