Instruction for Use

【Product Name】

Thyroid Peroxidase Antibody (Electrochemiluminescence Immunoassay)

【Order Information】

REF NO.	Package Size
690039	50T
690041	2×50T
690040	100T
690042	2×100T

【Intended Use】

Immunoassay for in vitro quantitative determination of antibody to thyroid peroxidase in human serum and plasma. Clinically, it's mainly used as an aid in diagnosis of hashimoto's thyroiditis and exophthalmic goiter.

Thyroid peroxidase (TPO), a membrane-bound glycoprotein containing heme prosthetic group, is the key enzyme in thyroid hormone biosynthesis process, which has special immunological characteristics and is the main effective antigen of thyroid microsomes[1]. TPO exists in the microsomal on thyroid cells, located at the apical plasma menbrane of the follicular epithelial cells. Normally, TPO doesn't enter the peripheral blood. when thyroid lesions occur and follicular cell damages, TPO is insulated from the follicular cavity edge spilled to peripheral blood, resulted to the body's immune disorder, Ts cell defects, Ts cell function decline, Th cell activity strengthening, and B lymphocytes into plasma cells to produce thyroid peroxidase antibody (anti-TPO). Combining anti-TPO with the TPO of normal follicular cells on the surface by complement C3 activation pathway and antibody dependent cell-mediated cytotoxicity, participates in follicular cell damage in hashimoto's disease, and causes thyroid immune damage. Anti-TPO is widely in autoimmune thyroid diseases, and the titer of anti-TPO is closely related to the cytotoxic effect of hashimoto's thyroid[2]. Anti-TPO is an significant immunological marker in patients with autoimmune thyroiditis (ATD). In patients with ATD, the concentration of anti-TPO is significantly increased, especially in hypothyroidism of hashimoto. In ATD, the change of anti-TPO was significantly correlated with thyroid microsomal antibody, but the determination of anti-TPO was more accurate and sensitive than thyroid microsomal antibody[3].

【Test Principle】

Competition principle. Total duration of assay: 18 minutes.

20 µL of sample was incubated with biotinylated TPO. After addition of anti-TPO-antibodies labeled with a ruthenium complex and streptavidin-coated microparticles, the anti-TPO antibodies in the sample compete with the ruthenium-labeled anti-TPO antibodies for the biotinylated TPO antigen. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is 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 Component】

The reagent pack consists of MB, RA, RB, calibrators and control materials (Optional). Different batch of reagent components should not be used interchangeably.

Component	Ingredients	Volume (50T)	Volume (2×50T)	Volume (100T)	Volume (2×100T)
Magnetic Beads (MB)	Streptavidin-coated microparticles 0.75 mg/mL: 0.1 M PBS; preservative	1×1.8 mL	2×1.8 mL	1×3.5 mL	2×3.5 mL
Reagent B (RB)	Biotinylated TPO, 0.25 mg/L; 0.05 M HEPES; preservative	1×3.8 mL	2×3.8 mL	1×7.5 mL	2×7.5 mL
Reagent A (RA)	Ru complex-labeled Anti-TPO, 0.1 mg/L; 0.05 M HEPES; preservative	1×3.5 mL	2×3.5 mL	1×7.0 mL	2×7.0 mL
Calibrators	Bovine serum albumin, preservative	High: 1×1.0 mL Low: 1×1.0 mL			
Control Materials (Optional)	Bovine serum albumin, preservative	High: 1×1.0 mL Low: 1×1.0 mL			

Traceability:This method can be traced back to NIBSC 66/387.

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 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 320510001, 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.

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

The expiration date is labeled on the box, rackpack and bottles.

Damaged, expired or contaminated reagents should be discarded.

【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】

Human serum and plasma added with heparin lithium, heparin sodium and EDTA-K3 are recommended. Blood samples should be collected by standard operation of venous puncture; after complete coagulation, the tangible component should be removed by centrifugation. The sample should avoid bubbling during testing. Lipid layer floating on the upper of the sample should be removed. Samples with severe hemolysis are not recommended. Samples would better to be tested in 8 hours after collection, otherwise, should be stored 2~8ºC for no more than 3 days or -20ºC, 1 month. Freezing and thawing cycle is permitted only once.

【Assay Procedure】

Testing procedures and precautions

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

Set up Thyroid peroxidase antibody (Anti-TPO) test according to the operational manual.

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

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 (RFID) reagent card (refer to instrument operational 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 28 days;

(3) quality control misses the target.

Quality control

For quality control, use Anti-TPO control materials.

In addition, other suitable control material can be used.

Control materials 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/mL or kIU/L. 1IU/mL=1kIU/L.

Dilution

Samples with anti-TPO concentrations above the measuring range can be diluted manually with anti-TPO sample diluent. The recommended dilution ratio is 1:5. The concentration of diluted sample must be >200 IU/mL. After dilution, the result by the dilution factor.
Note: Because self antibodies are heterogeneous, some individuals exhibit nonlinear dilution.

【Biological reference interval】

According to 275 cases (male, 132; female, 143) of human serum samples, the 97.5th percentile draws a reference range for males and females was <32.98 IU/mL. Each laboratory should investigate transferability of the reference range to its local population and if necessary determine its own reference ranges.

【Result Interpretation】

In interpreting the results, the patient's overall clinical situation should be referred to, including symptoms, medical history and other relevant data and information.

【Limitations】

Test results are used only for clinical reference and cannot be used as the basis for diagnosis or rule-out of diseases alone.
The measuring range of the kit is 10~600 IU/mL. Values below lower detection limit are reported as < 10 IU/mL. Samples with anti-TPO concentrations above the measuring range can be diluted with Anti-TPO sample diluent.
When samples contain the bilirubin concentration of 87.5 mg/dL or less, triglyceride concentration of 2500 mg/dL or less, concentration of biotin 30 ng/mL or less, hemoglobin concentration of 0.5g/dL or less, Total proteins concentration of 125 mg/mL or less, HAMA concentration at 361.81 ng/mL or less, the interference bias of determination results deviation within ±10%. The kit is undisturbed by rheumatoid factor (415.8 IU/mL).

【Analytical Performance】

Lower limit of measurement

Limit of Detection ≤10 IU/mL.

Accuracy

The relative bias of measuring international standard products within ±10%.
The relative bias of measuring trueness control materials within ±10%.

Linearity

The correlation coefficient (r) was not less than 0.9900 in the interval of 10~600 IU/mL.

Within-run precision (repeatability)

The coefficient of variation (CV) ≤ 7.5%.

Between-lot precision

The coefficient of variation (CV) ≤ 10%.

Analytical specificity

Test the concentration of 1000 IU/mL Anti-TG, the results were lower than 10 IU/mL.

Homogeneity of Calibrators and Control Materials

Within-bottle homogeneity

The coefficient of variation (CV) ≤ 10%.

Between-bottle homogeneity

The coefficient of variation (CV) ≤ 10%.

Method comparison

A comparison of the Lifotronic Anti-TPO assay (y) with the Roche Elecsys Anti-TPO method (x) using clinical samples gave the following correlations:
Number of samples measured: 198
Linear regression:
y=1.0034x+1.3026
r = 0.9934
The sample concentrations were between approx 9.86 and 562.1 IU/mL.

【Precaution and Warning】

The kit is only used for in vitro diagnostics.
When using the kit, it's necessary to comply with regulations in the laboratory.
Test results of the kit are for clinical reference only, and clinical evaluation of patients should be combined with their symptoms and signs, medical history, other laboratory examination results and treatment responses.
Due to factors such as methodology or antibody specificity, testing identical samples with reagents from different manufacturers may get different results, and the results from different kits should not compare directly, lest cause the wrong medical explanation; It's suggested that laboratorians should point out the characteristics of used reagent in the test report to the clinician. In serial monitoring, if the reagent type is changed, a continuous parallel test should be performed and comparison of the results to the former to determine new baseline value.
This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to 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.

【Symbols Explanation】

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

【Reference】

1.Czarnocka B, Ruf J, Ferrand M. Purification of the human thyroid peroxidase and identification as the microsomal antigen in thyriod disease. FEBS Lett, 1985;109-147.
2.Bogner U, Schleusener H, Wall JR. Antibody-dependent cell mediated cytotoxity against human thyroid cells in hashimoto's thyroiditis but not Graves'disease. J Clin Endocrinol Metab, 1984,59:734-740.
3.Feldt RU,Hemaden M, Semple GW, et al. Anti-thyroid peroxidase antibodies in thyroid disorders and nonthyroid autoimmune disease. Autoimmunity, 1999, 3(1):245.

【Manufacturer】

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