



Antibodies to Thyroid Peroxidase Quantitative Detection KIT (Chemiluminescent Immunoassay)

GIÁM ĐỐC
LIANG JIANGMING

REF	Packing Size
KH-G-L-335-01-CE	50 Tests/Kit Reagent 1:1×2.5mL, Reagent 2:1×11.0mL, Calibrator A: 1.0mL×1, Calibrator B: 1.0mL×1, Calibrator C: 1.0mL×1, Calibrator D: 1.0mL×1, Calibrator E: 1.0mL×1, Calibrator F: 1.0mL×1
KH-G-L-335-02-CE	100 Tests/Kit Reagent 1:1×5.0mL, Reagent 2:1×22.0mL, Calibrator A: 1.0mL×1, Calibrator B: 1.0mL×1, Calibrator C: 1.0mL×1, Calibrator D: 1.0mL×1, Calibrator E: 1.0mL×1, Calibrator F: 1.0mL×1
KH-G-L-335-03-CE	200 Tests/Kit Reagent 1:2×5.0mL, Reagent 2:2×22.0mL, Calibrator A: 1.0mL×1, Calibrator B: 1.0mL×1, Calibrator C: 1.0mL×1, Calibrator D: 1.0mL×1, Calibrator E: 1.0mL×1, Calibrator F: 1.0mL×1
KH-G-L-335-04-CE	100 Tests/Kit Reagent 1:1×10.0mL, Reagent 2:1×25.0mL, Calibrator A: 1.0mL×1, Calibrator B: 1.0mL×1, Calibrator C: 1.0mL×1, Calibrator D: 1.0mL×1, Calibrator E: 1.0mL×1, Calibrator F: 1.0mL×1

Intended Use

The Antibodies to Thyroid Peroxidase Quantitative Detection KIT is a chemiluminescent immunoassay for the quantitative determination of antibodies to thyroid peroxidase(Anti-TPO) in human serum ,and used for clinical auxiliary diagnosis.

Summary and Explanation

TPO, a glycosylated membrane protein, is on the upper surface of thyroid follicular cells ^[1]. Human TPO consists of 933 amino acids, of which 845 are extracellular amino acids, 60 are intracellular amino acids and a few amino acids form transmembrane part ^[2]. TPO plays an important role in the synthesis of thyroid hormones in human body, as it can catalyze both iodination of tyrosine residues in thyroglobulin, and the synthesis of two tyrosine residues in thyroglobulin into T4 and T3 ^[3].

TPO is a potential antigen, and TPO autoimmune antibody (Anti-TPO) can be detected in almost all hashimoto thyroiditis (HT) patients and 75% Graves disease (GD) patients ^[4]. High concentration of TPO antibody is a sign of disease. The sensibility can be improved if anti-TPO and anti-TSH receptor antibody are detected at the same time, but a negative result cannot exclude the possibility of autoimmunity disease ^[5].

Principle of Assay

Competitive protein-binding assay (CPBA) is applied. Add sample, TPO antigen-coated magnetic particles and TPO antibody alkaline phosphatase cross-linked polymer into reaction cup, and then incubate and wash. TPO antibody and TPO antibody alkaline phosphatase cross-linked polymer of the sample bind to TPO antigen captured on magnetic particles competitively. Add chemiluminescent substrate, and test chemiluminescence signal, which is measured in RLU. There is a functional relation between TPO antibody concentration of the sample and the RLU tested by instrument, and its concentration value is obtained by automatic calculation.

Kit Contents

Components	Main components	Unico	Polaris		
		100 tests/kit	50 tests/kit	100 tests/kit	200 tests/kit
Reagent 1	TPO antigen-coated magnetic particles, gelatin, buffer solution, preservative	10.0mLx1 vial	2.5mLx1 vial	5.0mLx1 vial	5.0mLx2 vial
Reagent 2	Alkaline phosphatase-labeled TPO antibody, gelatin, casamino acid, buffer solution, preservative	25.0mLx1 vial	11.0mLx1 vial	22.0mLx1 vial	22.0mLx2 vial
Calibrator A	Horse serum, buffer solution, preservative	1.0mLx1 vial	1.0mLx1 vial	1.0mLx1 vial	1.0mLx1 vial
Calibrator B	TPO antibody (15~35IU/mL), horse serum, buffer solution, preservative	1.0mLx1 vial	1.0mLx1 vial	1.0mLx1 vial	1.0mLx1 vial
Calibrator C	TPO antibody (35~75IU/mL), horse serum, buffer solution, preservative	1.0mLx1 vial	1.0mLx1 vial	1.0mLx1 vial	1.0mLx1 vial
Calibrator D	TPO antibody (100~200IU/mL), horse serum, buffer solution, preservative	1.0mLx1 vial	1.0mLx1 vial	1.0mLx1 vial	1.0mLx1 vial
Calibrator E	TPO antibody (320~480IU/mL), horse serum, buffer solution, preservative	1.0mLx1 vial	1.0mLx1 vial	1.0mLx1 vial	1.0mLx1 vial
Calibrator F	TPO antibody (480~720IU/mL), horse serum, buffer solution, preservative	1.0mLx1 vial	1.0mLx1 vial	1.0mLx1 vial	1.0mLx1 vial

The calibrators can be traced to WHO international standard substance (66/387). The definite value is featured with lot specificity. For such specific value, see the “calibrator” menu on instrument operation interface upon loading the lot information of the kit.

Note: Do not interchange or mix the components in test kits from different batches.

Materials Required but Not Provided

1. Cleaning solution
2. Substrate solution for full-automatic immunology test system
3. Reaction cup (for Polaris V150)
4. Washing solution
5. Reaction cup/suction tip (sets) (for Polaris i2400)
6. Quality control materials provided by Shanghai Kehua Bio-engineering Co., Ltd. (KHB) or other companies.

Note: Corresponding quality control concentration range shall be verified and set by the laboratory.

Storage

Unopened kits should be stored at 2 ~ 8°C and avoid freezing. The shelf life is 12 months.

Opened kits should be stored at 2 ~ 8°C and avoid freezing. It is recommended to use the kits within 28 days.

Please see the label for detailed manufacture date and expiry date.

Applicable Instrument

Automatic Chemiluminescence Immunoassay Analyzer: Polaris i2400, Polaris V150.

Sample Requirements

1. It is applicable for serum samples.
2. Please separate serum sample from blood collection tube and store them timely after sampling. Samples can be stored for two weeks at 2 ~ 8°C, and 6 months below -20°C. The samples cannot be frozen and thawed more than once.
3. If continuous monitoring is required for TPO antibody concentration of the same patient at different periods, the same type of blood collection tube from the same manufacturer shall be used.

4. *The samples containing precipitate or flocculus shall be centrifuged before test, and take supernatant for testing after centrifugation; it is recommended to centrifuge at 10,000g for 10mins.*
5. *Make sure there is no bubble for all samples to be tested before testing, otherwise it will affect test result..*

Testing Methods**● Sample preparation:**

1. *Each sample size for a single determination shall not be lower than 280 μ L, and 80 μ L of sample shall be added for each determination added.*
2. *Sample, calibrator and quality control are balanced to room temperature before use, frozen samples shall be tested after thawing and fully mixing.*
3. *Samples, calibrator and quality control after balance are placed on corresponding tube rack, and barcode shall not be covered when placing.*

● Reagent preparation:

1. *Check components of all reagent bottles are correct.*
2. *Whether the height of reagent bottles on reagent shelf is consistent, to prevent instrument from being stuck during transportation.*
3. *If the kit is tested for the first time, the barcode on the reagent shelf shall be scanned and aluminum foil films of all reagent bottles are torn.*
4. *The test can be carried out when putting reagent on reagent disks and fully mixing R1.*

● Test: *Choose Anti-TPO test procedure, and press "Run". After the instrument starts, the result will be tested automatically.*

● Calibration and quality control**1. Calibration**

- 1) *When conducting calibration, test calibrator A-F for 3 times each, and the instrument will generate corresponding calibration curves automatically.*
- 2) *Please test TPO antibody quality control while testing calibrator A-F, and calibration curve is effective when the value of quality control is within allowable range.*
- 3) *Recalibration shall be conducted under the following conditions:*

Use of test kit from a new batch;

Use of reagent from the same kit for more than 7 days;

Use of reagent from the same batch for more than 28 days;

Tested value of quality control is out of allowable range;

Changes of hardware and software including instrument repair and software upgrading.

2. Quality control:

- 1) *It is recommended to use the quality control materials provided by KHB or other companies such as BIO-RAD. Other commercial quality controls containing TPO antibody are also available, but corresponding quality control concentration range shall be set by the laboratory.*
- 2) *It is recommended to retest quality control if test interval is more than 24h.*

● Result calculation:

The instrument calculates TPO antibody concentration of the sample automatically, with unit of IU/ml.

● Sample dilution:

The sample cannot be diluted and the autoantibody shows heterogeneity, therefore non-linear dilution appears.

Reference Range

We tested 175 fasting serum samples of healthy people in Shanghai, and adopted percentile method for statistical

analysis. The results are showed in table below:

Sample size	Median (IU/ml)	99% percentile ranges of concentration (IU/ml)
175	16.09	44.50

The normal reference range we got is lower than 44.50IU/mL. However, considering the differences of region, gender and age, we also suggest each laboratory establish its own reference range.

Interpretation of Test Results

1. The test result is effective if the test result of quality control is within the allowable range; the test result is invalid if the test result of quality control falls outside the allowable range, then a calibration shall be set again for testing.

2. The detection range of this test kit is 7.5 ~ 600 IU/ml.

When the concentration is higher than 600 IU/ml, the result is displayed as ">600 IU/ml"; when the concentration is lower than 7.5IU/ml, the result is displayed as "<7.5IU/ml".

3. The review and analysis of test result shall be assumed by professionals. Clinicians shall provide comprehensive diagnosis according to the patient's physical sign, medical history and relevant indicators. If the test result is not in line with or even inconsistent with the clinical situation, analysis shall be conducted to find out the reasons.

Limitations of Testing Methods

1. The test result of the product is only for clinical reference and is not the only basis for clinical diagnosis and treatment. The clinical management of patients shall be subject to comprehensive consideration based on such patient's symptom/physical sign, other laboratory examinations (especially the etiology test), treatment reaction, epidemiology and other information.

2. Patients who have long been exposed to animals or animal serum products may have heterophil antibody. This antibody would react with immunoglobulin, thus interfering the test result. Specific analysis according to the patient's condition is therefore recommended.

3. Patients treated with Monoclonal antibodies (mAb) might get interfered TPO antibody test result. For samples of such patient, specific analysis according to clinical symptoms is therefore recommended.

4. Patients with hemolysis or lipemia might get inaccurate test results.

Performance Characteristics

1. Limit of detection: No higher than 7.5IU/mL .

LOT	lot 1	lot 2	lot 3
S1	3800817	3891932	3857471
S2	2962345	3016334	3017514
S1 mean RLU	3895514	3893631	3892175
SD _{S1}	44279	116449	53403
M _{S1} +2SD _{S1}	3806956	3660733	3785369
Limit of detection	0.00	6.60	2.15

2. Accuracy: The recovery of the kit is 85% ~ 115%.

	lot 1	lot 2	lot 3
Sample	Observed (IU/mL)	Observed (IU/mL)	Observed (IU/mL)
Sample A	429.8	463.4	453.3
Sample B	117.0	132.5	122.7
Sample C	145.1	159.8	150.5

Recovery %	92.6%	87.5%	88.4%
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3. Within-batch precision: CV ≤ 8%; Between-batch precision: CV ≤ 15%.

Test both samples for 10 times each to get Ms and SDs from the tested results, and calculate CV by the equation of $CV=SD/M \times 100\%$.

(1) Within-batch precision

LOT	lot 1			lot 2			lot 3		
Sample	M (IU/mL)	SD	CV	M (IU/mL)	SD	CV	M (IU/mL)	SD	CV
L	230.4	4.21	1.8%	246.9	4.09	1.7%	234.4	6.09	2.6%
H	549.0	13.78	2.5%	564.3	11.17	2.0%	578.8	14.96	2.6%

(2) Between-batch precision

Sample	M (IU/mL)	SD	CV
Sample H	564.0	17.92	3.2%

4. Linearity: Within concentration range of 7.5 ~ 600IU/ml, linearly dependent coefficient $r \geq 0.99$.

LOT		lot 1		lot 2		lot 3	
Sample	Dilution	Observed (IU/mL)	Linear correlation coefficient r	Observed (IU/mL)	Linear correlation coefficient r	Observed (IU/mL)	Linear correlation coefficient r
H1	1%	2.87	0.99801	3.27	0.99930	4.16	0.99744
H2	5%	17.17		22.98		20.6	
H3	10%	43.1		50.3		41.57	
H4	20%	97.7		114.1		98.9	
H5	60%	373.5		392		378	
H6	80%	533.4		547.9		543.6	
H7	100%	701.4		701.4		721	

5. Specificity

(1) Cross-reactivity

Interference substance	Amount Added	Cross-reactivity %		
		lot 1	lot 2	lot 3
Anti-TG	400IU/mL	0.6%	0.9%	-0.7%

(2) Interfering Substances

Interference substance	Maximum Amount Added	lot 1		lot 2		lot 3		Whether there is interference
		Recovery %	Recovery %	Recovery %	Recovery %	Recovery %	Recovery %	
Hemoglobin (Hb)	1000 mg/dL	99.29%	103.16%	98.43%	103.72%	101.70%	92.97%	no
Bilirubin	60 mg/dL	98.11%	99.89%	109.51%	91.30%	94.74%	92.61%	no
Chylomicron	0.50%	87.30%	108.67%	99.13%	98.21%	105.97%	103.45%	no
RF	250 IU/mL	106.50%	92.61%	100.97%	103.20%	95.62%	99.87%	no
ANA	160 titer	85.98%	93.55%	103.02%	91.37%	105.85%	121.20%	no

The interfering substances experiment showed that no interference was found with 1000 mg/dL of hemoglobin, 60 mg/L of bilirubin, 0.5% of chylomicron, 250 IU/mL of RF, 160 titer of ANA and 400IU/mL of Anti-TG .

Warnings and Precautions

Warning	
H300	Fatal if swallowed.
H302	Harmful if swallowed.
H310	Fatal in contact with skin.
H314	Causes severe skin burns and eye damage.
H330	Fatal if inhaled.
H373	May cause damage to organs through prolonged or repeated exposure if swallowed.
H400	Very toxic to aquatic life.
H410	Very toxic to aquatic life with long lasting effects.
Prevention	
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P262	Do not get in eyes, on skin, or on clothing.
P264	Wash skin thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P273	Avoid release to the environment.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 + P310	IF ON SKIN: Wash with plenty of water. Immediately call a POISON CENTER/ doctor.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.

1. For in vitro diagnostic use.
2. For professional use only. The product is to be used by personnel specially instructed and trained in the in vitro diagnostic procedures only.
3. Do not use the product if package is damaged.
4. Ensure the Reagent 1 is properly mixed before use.
5. Testing TPO antibody by different methods may get varying results. When continuous monitoring of the TPO antibody concentration is required, kits produced by the same manufacturer are recommended.
6. Avoid bubbles in the sample and reagents during operation. Any bubble shall be removed by clean cotton swab or pipette.
7. The operating environment and operator protection are explicitly described in the Instruction for Use. Since the testing object is human serum samples that may contain pathogenic pathogens, operators shall observe the requirements of infectious disease laboratory test.
8. All waste generated in the laboratory shall be disposed of in accordance with the "Laboratories - General requirements for biosafety" (GB 19489-2008) and the "Regulation of disinfection technique in healthcare settings" (WS/T 367-2012). Infectious wastes include unneeded samples, cultures and other articles. Such articles should be sealed in special leak-proof containers, and properly transported to the disinfection room, where they would be treated or discarded after high-pressure disinfection.
9. Some components of the test kit contain bovine-derived material BSA and horse-derived material horse serum, which comes from the national approved supply. However, according to GSYJX [2006] No. 407 "Notice on Registration of Medical Devices Containing Bovine and Sheep Derived Materials", there are still potential risks for transportation

personnel and users of BSA and horse serum. Relevant personnel shall take necessary safety measures. Direct contact of reagent with skin shall be avoided.

10. The reagent contains preservative sodium azide. Please do not swallow it. Direct contact with skin and mucosa shall be avoided. In case of accidental contact, wash with abundant water. In case of misusing, please go to the hospital immediately.

References

[1] Hobby Pet al. Identification of an immunodominant region recognized by human autoantibodies in a three-dimensional model of thyroid peroxidase. *Endocrinology*. 2000, 141(6):2018-2026.










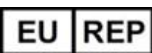






[2] Gardas A et al. Human thyroid peroxidase: mapping of autoantibodies, conformational epitopes to the enzyme surface. *Redox Rep*. 2000;5(4):237-241.

[3] Kimura S et al. Human thyroid peroxidase: complete cDNA and protein sequence, chromosome mapping, and identification of two alternately spliced mRNAs. *Proc Natl Acad Sci U S A*. 1987 ;84(16):5555-5559.

[4] Ludgate M et al. Antibodies to human thyroid peroxidase in autoimmune thyroid disease: studies with a cloned recombinant complementary deoxyribonucleic acid epitope. *J Clin Endocrinol Metab*. 1989 ;68(6):1091-96.

[5] Libert F et al. Thyroperoxidase, an auto-antigen with a mosaic structure made of nuclear and mitochondrial gene modules. *EMBO J*. 1987 ;6(13):4193-96.

Key symbols used

	Manufacturer		Temperature limit
	Batch code		Date of manufacture
	In vitro diagnostic medical device		Consult instructions for use or consult electronic instructions for use
	Catalogue number		Do not use if package is damaged
	Upward		Authorized representative in the European Community
	Use-by date		CE marking
	Contains sufficient for <n> tests		Biological risks
	Reagent 1		Reagent 2

CAL A	<i>Calibrator A</i>	CAL B	<i>Calibrator B</i>
CAL C	<i>Calibrator C</i>	CAL D	<i>Calibrator D</i>
CAL E	<i>Calibrator E</i>	CAL F	<i>Calibrator F</i>
CAL A-F	<i>Calibrator A, Calibrator B, Calibrator C, Calibrator D, Calibrator E, Calibrator F</i>		



*Shanghai Kehua Bio-engineering Co., Ltd.
 1189 North Qinzhou Road, 200233 Shanghai, PEOPLE'S REPUBLIC OF CHINA
 Tel: +86-21-64950625
 E-mail: international@skhb.com
 Website: www.skhb.com/en*



*QbD RepS BV
 Groenenborgerlaan 16
 2610 Wilrijk
 Belgium*