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## ANTI-THYROGLOBULIN (ANTI-TG) CHEMILUMINESCENCE

### IMMUNOASSAY KIT

Catalog No. CL1006-2

#### **INTENDED USE**

The Autobio anti-thyroglobulin (anti-Tg) chemiluminescence immunoassay (CLIA) kit is intended for the quantitative determination of Anti-Tg concentration in human serum.

#### **INTRODUCTION**

The quantitative determination of thyroglobulin (Tg) autoantibodies in human serum or plasma with a microtiter plate chemiluminescence immunoassay. Measurements of Tg autoantibodies may aid in the diagnosis of certain thyroid diseases such as Hashimoto's and Grave's as well as nontoxic goiter<sup>1,2</sup>. Antibodies to thyroglobulin have been shown to be characteristically present from patients with thyroiditis and primary thyrotoxicosis<sup>3,4</sup>. This has led to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction. Passive Hemagglutination (PHA) methods have been employed in the past for measurements of antibodies to Tg<sup>5,6</sup>. However, PHA tests do not have the sensitivity of enzyme immunoassay and are limited by subjective interpretation. This product, with the enhanced sensitivity of chemiluminescence Immunoassay, permits the detection of subclinical levels of antibodies to Tg. In addition, the results are quantitated by a Luminometer, which eliminates subjective interpretation.

#### **PRINCIPLE OF THE TEST**

The Anti-Tg CLIA test is a solid phase two-step immunoassay. Tg Antigen is coated on the surface of the microtiter wells and reference standard, diluted patient specimen, and control sera are added to their respective wells. Then autoantibodies to Tg present in the added reagents bind to Tg antigens and form an immune complex, which is deposited to the surface of coated wells. After the completion of the required incubation period, aspiration or decantation removes the reactants that are not attached to the wells. An enzyme anti-human IgG conjugate is then added to permit quantitation of reaction through interacting with human IgG in the immune complex. After washing, the unbound antibody-enzyme conjugates are removed. The horseradish peroxidase activity bound in the wells is then assayed by a chemiluminescence reaction. The Related Light Unit (RLU) of the reaction is proportional to the concentration of Anti-Tg present in the sample.

#### **MATERIALS PROVIDED**

1. Antigen Coated Microtiter Plate: Microplate with thyroglobulin (Tg) coated wells (1 plate, 48 wells/96wells)
2. Enzyme Conjugate Reagent: Horseradish Peroxidase (HRP) labeled Anti-Human IgG in Stabilizing Buffer (1 vial, 6.0ml/11.0 ml)
3. Reference Standards: 5, 62.5, 250, 500, 1000, 2000IU/ml Anti-Tg in Tris solution with preservatives (6 vials, lyophilized)
4. Sample diluent: (1 vial/2vials, 50ml/ea)
5. Substrate A: (1 vial, 3.5ml/6.0ml)
6. Substrate B: (1 vial, 3.5ml/6.0ml)
7. PBS-T Powder: PBS-Tween (1 bag, 5g)

#### **MATERIALS NOT PROVIDED**

The following materials are required but not provided in the kit.

1. Distilled water
2. Precision pipettes for delivery of 20-200 $\mu$ l, 100-1000 $\mu$ l (the use of accurate pipettes with disposable plastic tips is recommended)
3. Luminometer
4. Vortex Mixer or equivalent
5. Washer for microplate
6. Quality control specimens
7. Incubator
8. Absorbent paper

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**STORAGE OF TEST KIT AND INSTRUMENTATION**

1. Unopened test kits should be stored at 2~8°C upon receipt. The test kit may be used throughout the expiration date of the kit (1 year from the date of manufacture). Refer to the package label for the expiration date.
2. Reconstituted reference standards should be used within 30 days and be frozen at -20°C for long term storage. Microplate after first using should be kept in a sealed bag with desiccants to minimize exposure to damp air. Other opened components will remain stable for at least 2 months, provided it is stored as prescribed above.

**SPECIMEN COLLECTION AND PREPARATION**

1. Serum is the recommended sample type for this assay. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Allow samples to clot for 1 hour before centrifugation.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Prior to use, specimens should be capped and stored up to 48 hours at 2~8°C. For longer storage, freeze the specimens at -20°C. Thawed samples must be mixed prior to testing.

**PRECAUTIONS AND WARNINGS**

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where bovine spongiform encephalopathy(BSE) has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

**REAGENT PREPARATION**

1. All reagents should be brought to room temperature (18~25°C) prior to use.
2. Adjust the incubator to 37°C
3. Reconstitute each lyophilized reference standard with 1.0ml of distilled water. Allow the reconstituted material to stand for at least 10 minutes. Reconstituted reference standards should be stored sealed at 2~8°C.
4. Prepare Wash Solution: Add the whole bag of PBS-T Powder to 500 ml of distilled water and mix well. The wash solution is stable at room temperature for 2 months.
5. Sample Dilution (1:100): Dispense 0.01ml (10µl) of each specimen into 1ml of Sample diluent. Cover and vortex or mix thoroughly by inversion. Store at 2~8°C for up to 48 hours. Reference standards and control sera need not to be diluted.

**IMPORTANT NOTES**

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. It is recommended that no more than 32 wells be used for each assay run, if manual pipette is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipette is available.
4. Replace caps on reagents immediately. Do not switch caps.
5. The wash procedure is critical. Insufficient washing will result in poor precision and invalid results.

**ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
2. Dispense 100µl of standards, diluted samples, and controls into appropriate wells.
3. Thoroughly mix for 30 seconds. It is important to have complete mixing in this step.
4. Incubate at 37°C for 30 minutes.
5. Remove the incubation mixture by flicking plate contents into a waste container.
6. Rinse and flick the microtiter plate 5 times with wash solution.
7. Strike the plate sharply onto absorbent paper to remove residual water droplets.
8. Dispense 100µl of Enzyme Conjugate Reagent into each well.

9. Incubate at 37°C for 30 minutes
10. Remove the incubation mixture by flicking plate contents into a waste container.
11. Rinse and flick the microtiter plate 5 times with Wash Solution.
12. Dispense 50µl of Substrate A, then 50µl of Substrate B into each well. Gently mix for 10 seconds.
13. Put the microtiter plate into the detecting chamber of Luminometer for 5 minutes, then read the RLU values of each well.

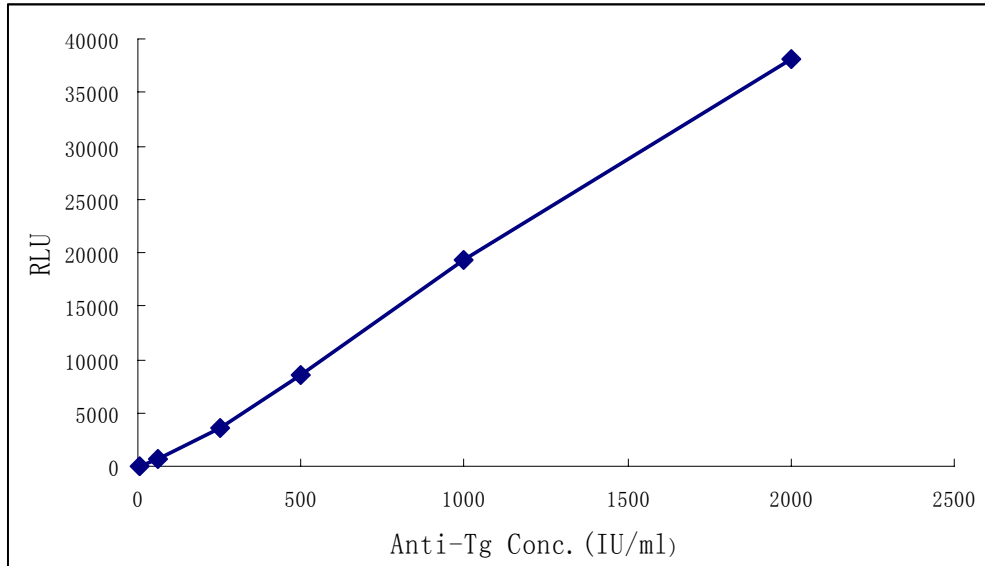
**CALCULATION OF RESULTS**

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. On linear graph paper plot the RLU (ordinate) for each reference standard against the corresponding concentration of Anti-Tg in IU/ml (abscissa) and draw a calibration curve through the reference standard points by connecting the plotted points with straight lines.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

**EXAMPLE OF STANDARD CURVE**

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

Anti-Tg (IU/ml)	RLU
5	19.209
62.5	728.265
250	3644.94
500	8500.33
1000	19291.5
2000	38091.6



**EXPECTED VALUES**

Human serum specimens were collected from a population of 147 healthy individuals in terms of TSH level. Values in excess of 110IU/ml are considered positive of anti-Tg. This normal range is suggested as a guideline. It is recommended that each laboratory establishes its own normal range.

**PERFORMANCE**

**A. Sensitivity**

20 zero standards were assayed along with a set of other standards. The sensitivity, defined as the apparent concentration corresponding to two standard deviations above the average RLU at zero binding, is lower than 10IU/ml.

### B. Specificity

The cross-reactivity of the Anti-Tg assay kit with Anti-TPO was determined by adding the interferent to zero standards. The RLU produced was then determined.

Interferent	Concentration	Measured Value (IU/ml)	Crosstalk Rate
Anti-TPO	500IU/ml	35	7%

### C. Precision

#### a. Intra-Assay Precision

Intra-Assay Precision was determined by assaying 20 replicates of each of 2 sera; low and high.

Serum	Number	Mean	SD	RSD (%)
Low	20	83.08	5.35	6.44
High	20	289.92	16.93	5.84

#### b. Inter-Assay Precision

Inter-assay Precision was determined by assaying duplicates of 2 serum pools in 20 separate runs, using a standard curve constructed for each run.

Serum	Number	Mean	SD	RSD (%)
Low	20	85.15	9.42	11.06
High	20	293.21	25.05	8.54

### D. Accuracy

For 134 samples in the range of 35IU/ml I to 1864IU/ml, the relationship between the Autobio Anti-Tg CLIA Test and the Abbott Architect® Anti-Tg (CMIA) assay is described by the equation below:

Reference	No. of Specimens	Least Square Regression Analysis	Correlation Coefficient
Abbott Architect Anti-Tg (CMIA)	134	$y = 1.0974x + 25.418$	0.963

### LIMITATIONS

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.






About 20% of asymptomatic specimens may present with anti-Tg autoantibodies reflecting the prevalence in apparently healthy populations. The prevalence of anti-Tg may also depend on age, gender, and geographic region of the selected population.




For diagnostic purposes, the results obtained from this assay should always be used in combination with clinical examinations, patient medical history, and other findings.

### QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

### SYMBOLS


	BATCH CODE
	USE BY
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <n> TESTS
	IN VITRO DIAGNOSTIC MEDICAL DEVICE

	<p><b>TEMPERATURE LIMITATION</b></p>
	<p><b>CATALOGUE NUMBER</b></p>
	<p><b>CONSULT INSTRUCTIONS FOR USE</b></p>

**REFERENCES**

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4. Surk M L, Chopra I J, Mariash C N. *et al.* American thyroid association guidelines for use of laboratory tests in thyroid disorders [J]. *JAMA*, 1990, 263: 1529-1532.
5. Schaadt B, Feldt-Rasmussen U, Rasmusson B, *et al.* Assessment of the influence of thyroglobulin (Tg) autoantibodies and other interfering factors on the use of serum Tg as tumor marker in differentiated thyroid carcinoma. *Thyroid* 1995; 5(3): 165-70.
6. McIntosh RS, Assh~MS, WeetmanAP. The antibody response in human antoimmune thyroid disease. *Clin Sci (Colch)*, 1997, 92: 529-541.

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