BioCLIA® Autoimmune Reagent Kit

Anti-TG

Chemiluminescent Microparticle Immunoassay

Magnetic bead chemiluminescence immunoassay (CLIA) for quantitative determination of anti-TG antibody in human serum

Key to Symbols Used

REF	Catalog Number		Expiration Date
IVD	For *In Vitro* Diagnostic Use	LOT	Lot Number
	Store between +2°C and +8°C		Consult Instruction for Use
	Manufacturer	EC REP	Authorized Representative in European Union
ANTIGEN BIOT	Biotinylated Antigen	Σ	Contains Sufficient for < *n* > Tests
CONJ AP ANTIGEN	Conjugate		Chemical Risk Warning
M STREP	Microparticle		Biological Risk Warning

HOB®

BioCLIA® Autoimmune Reagent Kit , Anti-TG

Intended Use

BioCLIA Anti-TG assay is intended for the in vitro quantitative measurement of anti-thyroglobulin antibodies (Anti-TG) in human serum as an aid in the diagnosis of Hashimoto's Thyroiditis, Graves' Disease or other related disorders in conjunction with other laboratory and clinical findings. This kit is used on the instrument of BioCLIA® 1200 and BioCLIA® 6500.

Catalog Numbers

MY00135 (50 Tests/kit)

MY00186 (100 Tests/kit)

Summary and Explanation

Thyroglobulin (Tg) is a large heterologous glycoprotein (660 kDa) exists in thyroid follicular cell, play an important role in the biosynthesis of thyroid hormone, T3 and T4. [1] In thyroid follicular cells, thyroid peroxidase catalyzes iodide reaction of tyrosyl group in Tg, and iodinated Tg will be kept in follicle colloid as a saver for T3 and T4. When the thyroid gland is stimulated, Tg will degrade and consequently the thyroid hormone, T3 and T4 release into blood. [2, 3]

The detection of anti-Tg antibodies is an effective method for diagnosis of autoimmune thyroid disease. Anti-Tg antibodies levels increase in 80-100% lymphoma thyroiditis or chronic thyroiditis cases, 10-20% subacute thyroiditis patients and 60-70% patients with hyperthyroidism. [4] It also related to patients with Addison's disease or Insulin-Dependent Thyroid disease (IDDM). Due to the heterogeneity of Tg, anti-Tg antibodies are also positive in elder patients with other diseases or people with normal thyroid function. [3, 4]

Principles of the Procedure

This kit is based on sandwich chemiluminescent microparticle immunoassay. The streptavidin coated magnetic beads, the biotinylated TG and human serum sample (containing anti-TG) are mixed in an assay tube, which allows anti-TG to bind to the beads surface. Then TG conjugated alkaline phosphatase (AP enzyme) is added and the bound enzyme is detected by addition of chemiluminescent substrate. The relative light unit (RLU) intensity is proportional to the amount of anti-TG.

For quantitation of anti-TG antibodies, the BioCLIA Anti-TG assay utilizes a predefined lot specific master calibration curve that is uploaded into the instrument through the reagent cartridge barcode. The master calibration curve is created during manufacturing by using in-house calibrators that are traceable to the Chinese national standard material (code: 150556). Based on the master calibration curve, and results obtained by running two calibrators, an instrument specific working curve is created, which is used to calculate International Units (IU)/mL from the RLU obtained for each sample.

Specimen Collection

The appropriate specimen types for BioCLIA Anti-TG are human serum. Cloudy samples should be purified by low-speed centrifugation. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increasing clotting time.

Freshly collected specimens stored in refrigerator (2-8 °C) are valid for testing within 8 days. The unopened specimen should reach to room temperature (18-25°C) before testing, and should not be stored in this temperature condition more than 2 days. All opened/on board specimens should be tested within 10 hours. Three freeze-thaw cycles for specimens do not affect the testing results.

Warnings and Precautions

1. This assay is only for use in the BioCLIA® 1200 and BioCLIA® 6500.

2. This product requires the handling of calibrators, controls and human specimens which contain human sourced materials. It is recommended that all human sourced materials are considered to be potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. 5 Biosafety Level 2 [6] or other appropriate biosafety practices [7, 8] should be used for materials that contain or possibly contain infectious agents. Avoid contacting with skin and eyes. Wear suitable protective gloves and clothing. Avoid contacting with skin and eyes. Wear suitable protective gloves and clothing.

3. Liquid waste and solid waste are temporarily stored at separate containers in the BioCLIA® 1200 and BioCLIA® 6500 in separate containers. Waste management should also be handled in accordance with standards mentioned in chapter Warnings and Precautions point No. 2.

4. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing wastes.

5. Once opened, this reagent cartridge must be stored in the instrument's reagent carousel. Avoid spilling the reagents when the reagent cartridge is placed into the instrument.

6. Chemical contamination of the reagents can resulting from improper cleaning or rinsing of the instrument. Residues from common laboratory chemicals such as formalin, bleach, ethanol, or detergent can cause interference in the assay. Be sure to follow the recommended cleaning procedure of the instrument as outlined in the BioCLIA® 1200 and BioCLIA® 6500 operator.

7. Precautions: 

Proclin 300 added in the kit reagents (Microparticle, TG Antigen, Conjugate) at concentration between 0.0015% - 0.6%.

Storage Instructions

The kit is stable until the expiration date, if it is stored and handled. Routine store the kit in refrigerator (2-8 °C). Vial opened reagents or onboard reagents can be used up to 28 running days (2-8 °C). The BioCLIA® 1200 and BioCLIA® 6500 software monitors the expiration of the reagent cartridge. The system will not accept expired reagents. Three freeze-thaw cycles before testing has no effect on the kit reagents.

Materials Supplied

Components are matched in sets. Labels supplied within the kit will be needed for the assay testing

- **TG Antigen** 1 bottle (5/10 mL) biotinylated TG antigen in 0.01 M PBS (pH7.4) buffer with stabilizer. Preservatives: 0.0015% < Proclin 300 < 0.6%. | TG | BIOT |
- **Conjugate** 1 bottle (6.75/13.5 mL) AP labeled TG antigen in 0.05 M MES (pH6.0) Buffer with stabilizer. | CONJ | AP | TG |
  Preservatives: 0.0015% < Proclin 300 < 0.6%.
- **Microparticle** 1 bottle (2.5/5 mL) Streptavidin-microparticles in 0.01 M PBS (pH7.4) buffer with stabilizer. | M | STREP |
  Preservatives: 0.0015% < Proclin 300 < 0.6%.

Kit Component Supplied Separately

Additional Materials Required But Not Provided:

- BioCLIA® 1200 (Cat No. MA00139)
- BioCLIA®6500 (Cat No. MA00243)
- BioCLIA Autoimmune Calibrator Set, Anti-TG
- (Cat No. MY00237, 6 x 1 mL;
  Cat No. MY00288, 12 x 1 mL)
- BioCLIA Autoimmune Control Set, Anti-TG
- (Cat No. MY00339, 2 x 1 mL;
- Cat No. MY00390, 4 x 1 mL)
- BioCLIA Sample Diluent I (Cat No. MY00965)
- BioCLIA System Wash Buffer (Cat No. MY00404)
- BioCLIA System Substrate (Cat No. MY00405)
- BioCLIA Cuvettes (Cat No. MA00138, MA00244)
- BioCLIA Silicon Gasket (Small) (Cat No. MV00195)
- BioCLIA Silicon Gasket (Large) (Cat No. MV00196)
- BioCLIA Substrate Tube Maintenance cleanser (Cat No. MA00140)
- BioCLIA Sample Probe Maintenance cleanser (Cat No. MA00141)
- BioCLIA Micro Cup (Cat No. MA00142)
- Distilled Water

Assay Procedure

Note that, it is important to perform all routine maintenance procedures for optimal performance, such as routine cleaning, calibration and control procedures that are defined in the BioCLIA® 1200 and BioCLIA® 6500 User Manual.

See the BioCLIA® 1200 and BioCLIA® 6500 User Manual for preparation, setup, dilutions, adjustment, assay and quality control procedures.

Users should have the periodic calibration procedure for every 28 running days from last calibration. Besides, a calibration procedure should be carried out when a new batch of BioCLIA Anti-TG kit is used.

The control procedure should be done before running the specimens each day. Users also can adjust the control procedure period according to their own lab frequency.

Expected Values

Each Laboratory should establish its own reference ranges.

When the customer see a problem (High CV or unusual values, rerun controls and analyze specimens again.

Result Analysis

With the help of the built in master calibration curve and specified two-point calibrator set for the instrument, the BioCLIA® 1200 and BioCLIA® 6500 will automatically calculate the Anti-TG concentration of each specimen and interpret the results into IU/mL.

Sample Dilution

No dilution is required.

Cut-Off Value Determination

This detection system is used for detecting sera from healthy people with normal Anti-TG range (n=233). 95% normal population lies in a reference range of ≤ 4.0 IU/mL.

Results may differ between laboratories due to variations in population and test method. Each laboratory should establish its own reference range.

Test Result Interpretation

Specimen with concentration ≤ 4.0 IU/mL, judge as healthy people;

Specimen with concentration > 4.0 IU/mL, judge as autoimmune thyroid disease.

Test results only reflect the sample collecting status and should be judged for diagnosis in conjunction with other laboratory and clinical findings.

Performance Characteristics

APPEARENCE

Kit components are complete with no leakage. No precipitation or floc in liquid reagents. Packing label should be clear and easy to be identified.

ACCURACY

This assay consists of two reference materials (low,

high) which are traceable to China National Standard Anti-Thyroglobulin Antibodies (Anti-TG), CNS Code: 150556. The reference materials are tested in triplicate to obtain a value of M, and calculated as: Measured deviation = (M - theoretical value)/ theoretical value x 100%.*

Samples (IU/mL)	Ave. Conc.	Exp.	Measured Deviation
Sample 1 (low)	9.72	10	-2.8%
Sample 2 (high)	1020.33	1000	2.0%

*Representative data; results in individual laboratories may vary from these data.

TRACEABILITY

Anti-TG antibody concentration can be traced to China National Standard Anti-Thyroglobulin Antibodies (Anti-TG), CNS Code: 150556.

PRECISION

A study based on guidance from (NCCLS) document EP-A[18] was performed for determining the precision of BioCLIA Anti-TG kit precision. Human sera in the in-house reference panel (RP1, RP2, RP3, RP4, RP5) tested with 10 replicates per sample for intra-assay precision evaluation, while with 4 replicates per sample for inter-assay precision. Each sample tested in individual runs, and 2 runs per day for 10 days, a total of 80 points. Data from this study are summarized in the following table.*

Intra-assay precision: CV ≤ 10%

Intra-Assay	RP1	RP2	RP3	RP4	RP5
Mean (IU/mL)	14.3	67.6	358.97	1133.55	1493.77
CV	3.32%	3.26%	5.75%	7.00%	4.24%

Inter-assay precision: CV ≤ 15%

Inter-Assay	RP1	RP2	RP3	PR4	RP5
Mean (IU/mL)	16.85	75.50	326.35	1041.16	1491.97
CV	6.20%	6.37%	9.81%	11.97%	5.89%

*Representative data; results in individual laboratories may vary from these data.

LIMIT OF BLANK / DETECTION (LOB/LOD)

LOB/LOD was determined consistent with CLSI EP17-A guideline. LOB/LOD of the BioCLIA Anti-TG assay was lower than 0.9 IU/mL, which is below the analytical measuring range of the assay.

ASSAY REPORTABLE RANGE

The BioCLIA Anti-TG has a reportable linear range of 0.9-2500 IU/mL, which covers the kit LoD to the highest point of Anti-TG in-house calibrators.

INTERFERENCE

Bilirubin, hemoglobin, triglycerides, rheumatoid factor (RF), and human anti-mouse antibody (HAMA) will not affect the BioCLIA Anti-TG assay performances when at the level indicated below.

Bilirubin ≤ 40 mg/dL;

Hemoglobin ≤ 500 mg/dL;

Triglycerides ≤ 3,000 mg/dL;

Rheumatoid factor (RF) ≤ 1,000 IU/mL;

Human anti-mouse antibody (HAMA) ≤ 2,000 ng/mL.

CROSS-REACTIVITY

The manufacturer performed testing to determine the cross reactivity of the assay to these substances:

Cross Reactant	Analyte Added (ug/mL)	Cross Reactivity (%)
Paracetamol	20 mg/dL	3.07%
Ibuprofen	50 mg/dL	2.56%
Aspirin	65 mg/dL	4.10%
Heparin sodium	47 mg/dL	0.96%

METHOD COMPARISON

Method comparison was implemented by comparing BioCLIA Anti-TG assay to the predicated assay.

Clinical Sample		BioCLIA Anti-TG		
		-	+	Total
Predicated Method	-	64	1	65
	+	0	35	35
	Total	64	36	100

Sensitivity	100.0%
Specificity	97.2%
Total agreement	99.0%

Limitations

- The effectiveness of this kit is only confirmed for human sera, the applicability of the other kinds of samples is not verified.
- Reliable and reproducible results will be obtained when the assay procedure is carried out in accordance with the instructions and with adherence to good laboratory practice.
- Clinical diagnosis should not be made on the findings of a single test result, but should be integrate with all clinical and laboratory findings.

References

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Laboratory Workers from Occupationally Acquired Infections: Approved Guideline - Third Edition. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute, 2005.

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Technical Assistance

For technical assistance, contact your National Distributor.

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