ab178631 – Anti-Thyroglobulin Human ELISA Kit

Instructions for Use

For the quantitative measurement of IgG class antibodies to Thyroglobulin in Human serum or plasma.

This product is for research use only and is not intended for diagnostic use.

Version 2 Last Updated 14 December 2015

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1. BACKGROUND

Abcam's anti-Thyroglobulin *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative determination of auto antibodies to thyroglobulin in Human serum or plasma.

A 96-well plate has been precoated with Human thyroglobulin to bind cognate antibodies. Controls or test samples are added to the wells and incubated. Following washing, a horseradish peroxidase (HRP) labelled anti-Human IgG conjugate is added to the wells, which binds to the immobilized thyroglobulin-specific antibodies. TMB is then catalyzed by the HRP to produce a blue color product that changes to yellow after adding an acidic stop solution. The intensity of yellow coloration is directly proportional to the amount of thyroglobulin sample captured in plate.

Thyroglobulin is a 660 kDa, dimeric glycoprotein produced by the thyroid gland and involved in the storage and synthesis of thyroid hormones. It is the precursor of the thyroid hormones 3,3',5-triiodo-L-thyronine (T_3) and L-thyroxine (T_4).

The enzyme thyroperoxidase (TPO) promotes iodination of tyrosine residues in thyroglobulin molecules, forming monoiodotyrosine (MIT) and diiodotyrosine (DIT). TPO further catalyzes the intramolecular coupling of two molecules of diiodotyrosine to produce tetraiodothyronine (T_4). The coupling of one monoiodotyrosine and one diiodotyrosine molecule results in triiodothyronine (T_3).

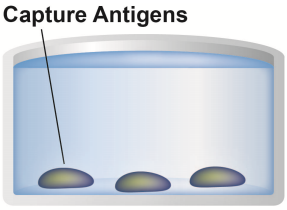
Thyroglobulin is a major autoantigen in autoimmune thyroiditis. Serum antibodies against thyroglobulin may be present at high concentrations in patients with Graves' disease and Hashimoto's thyroiditis.

Thyroglobulin auto antibodies are also found in some clinically euthyroid individuals, but usually at lower concentrations than in patients with clinical disease.

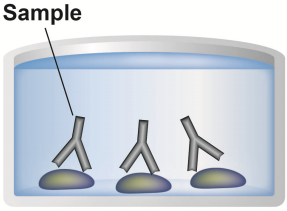
In addition to autoimmunity, anti-thyroglobulin antibodies may develop in patients suffering from thyroid cancer. High levels of anti-thyroglobulin may interfere with correct determination of serum thyroglobulin. Thyroglobulin is an established tumor marker for thyroid cancer, and is used post-surgically to evaluate the effectiveness of treatment and to monitor for disease recurrence.

Auto antigen	Disease	Sympotoms
Thyroglobulin	Hashimoto's thyroiditis	Initially hyperthyroidism: nervousness, weight loss, insomnia, fast heart rate, heat intolerance etc. Later gradual transition to chronic hypothyroidism: fatigue, decreased concentration, weight gain, constipation, cold intolerance etc.
	Graves' disease (= Morbus Basedow)	Hyperthyroidism: tachycardia, exophthalmos, struma (Merseburg triad)

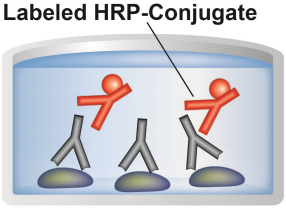
2. ASSAY SUMMARY



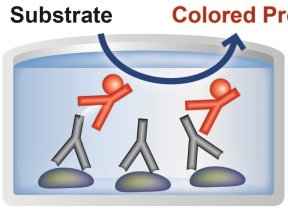
Prepare all reagents, samples and controls as instructed.



Add samples and controls to wells used. Incubate at 37ºC.



Wash each well and add prepared labeled HRP-Conjugate. Incubate at room temperature.



After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Thyroglobulin Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
IgG Sample Diluent***	100 mL	2-8°C
Stop Solution	15 mL	2-8°C
20X Washing Solution*	50 mL	2-8°C
Thyroglobulin anti-IgG HRP Conjugate**	20 mL	2-8°C
TMB Substrate Solution	15 mL	2-8°C
Thyroglobulin Negative control***	2 mL	2-8°C
Thyroglobulin Positive control***	2 mL	2-8°C
Thyroglobulin Standard A – 0 U/mL***	2 mL	2-8°C
Thyroglobulin Standard B – 2 U/mL***	2 mL	2-8°C
Thyroglobulin Standard C – 4 U/mL***	2 mL	2-8°C
Thyroglobulin Standard D – 8 U/mL***	2 mL	2-8°C
Thyroglobulin Standard E –32 U/mL***	2 mL	2-8°C
Thyroglobulin Standard F – 128 U/mL***	2 mL	2-8°C
Strip Holder	1 unit	2-8°C
Cover Foil	1 unit	2-8°C

* Contains 0.1 % Bronidox L after dilution

** Contains 0.2 % Bronidox L

*** Contains 0.1 % Kathon

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Incubator at 37°C
- Multi and single channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use

- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 **1X Washing Solution**

Prepare 1X Washing Solution by diluting 20X Washing Solution with deionized water. To make 200 mL 1X Washing Solution combine 10 mL 20X Washing Solution with 190 mL deionized water. Mix thoroughly and gently.

- All other solutions are supplied ready to use

10. SAMPLE COLLECTION AND STORAGE

- Use Human serum or plasma (citrate) samples with this assay. If the assay is performed within 5 days of sample collection, the specimen should be kept at 2-8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -80°C). If samples are stored frozen, mix thawed samples well before testing.

  Avoid repeated freezing and thawing.

  Heat inactivation of samples is not recommended

11. SAMPLE PREPARATION

- Before assaying, all samples should be diluted 1:100 with IgG Sample Diluent. Add 10 µL sample to 1 mL IgG Sample Diluent to obtain a 1:100 dilution. Mix gently and thoroughly.
- For samples with expected anti-thyroglobulin concentrations greater than Standard F (128 U/mL) a second 1:1 of this 1:100 diluted sample should be performed; e. g. 100 µL of first sample dilution + 100 µL of IgG Sample Diluent (mix well). Dilution factor: 2.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates)

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Reliability of results depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**
- **All controls (thyroglobulin positive and thyroglobulin negative) must be included with each assay performed to determine test results**

13.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3. Add 100 µL of each standard or sample into appropriate wells. Leave one well for substrate blank.

13.4. Cover wells with the foil supplied in the kit and incubate for 30 minutes at 37°C.

13.5. Remove the foil, aspirate the contents of the wells and wash each well three times with 300 µL of 1X Washing Solution. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 seconds. After the last wash, remove the remaining 1X Washing Solution by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.

Note: Complete removal of liquid at each step is essential for good assay performance.

13.6. Add 100 µL thyroglobulin anti-IgG HRP Conjugate into all wells except for the blank well. Cover with foil.

13.7. Incubate for 30 minutes at room temperature. Do not expose to direct sunlight.

13.8. Repeat step 13.5.

13.9. Add 100 µL TMB Substrate Solution into all wells

13.10. Incubate for exactly 15 minutes at room temperature in the dark.

13.11. Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.

Note: Any blue color developed during the incubation turns into yellow.

13.12. Highly positive samples can cause dark precipitates of the chromogen. These precipitates have an influence when reading the optical density. Predilution of the sample is recommended (See Section 11. Sample Preparation).

13.13. Measure the absorbance of the specimen at 450 nm within 30 minutes of addition of the Stop Solution.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

14. CALCULATIONS

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank:** Absorbance value **< 0.100**
- **Standard A:** Absorbance value **< 0.200**
- **Standard B:** Absorbance value **> Standard A**
- **Standard C:** Absorbance value **> Standard B**
- **Standard D:** Absorbance value **> 0.100**
- **Standard E:** Absorbance value **> 0.400**
- **Standard F:** Absorbance value **> 1.000**
- **Negative Control:** Absorbance value **< 6 U/mL**
- **Positive Control:** Absorbance value **> 12 U/mL**

Standard A < Standard B < Standard C < Standard D < Standard E < Standard F

If these criteria are not met, the test is not valid and must be repeated.

Calculation of Results

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in U/mL.

Readings of additionally (1+1) diluted patient samples must be multiplied by the appropriate dilution factor in order to obtain correct results! Dilution: 1+1 = Dilution factor: 2

Interpretation of Results

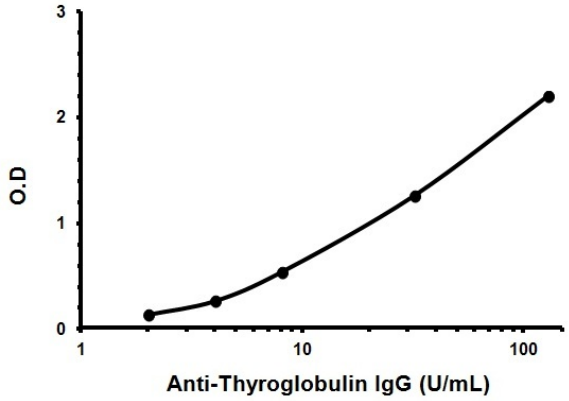
Normal value ranges for this ELISA should be established by each researcher.

The following values should be considered as a guideline only:

Normal	< 8 U/mL
Inconclusive (Grey zone):	8-10 U/mL
Non reactive:	> 10 U/mL

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



16. TYPICAL SAMPLE VALUES

PRECISION –

	Intra-Assay		Inter-Assay		
Standard	Positive serum	Positive serum	Positive serum	Positive serum	Negative serum
n=	20	20	12	12	12
Mean (OD)	1.812	0.697			
Mean (U/mL)			59.7	28.9	7.3
%CV	2.1	1.2	5.7	8.5	3.9

17. ASSAY ANALYTICAL SPECS

SPECIFICITY -

The specificity is 97.1 % and is defined as the probability of the assay scoring negative in the absence of the specific analyte.

SENSITIVITY -

The sensitivity is 87.5% and is defined as the probability of the assay scoring positive in the presence of the specific analyte.

The concentration of the analyte that can be distinguished from the zero calibrator is <0.5 U/mL.

18. INTERFERENCES

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

19. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time to short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

20. NOTES

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