

# **ab178632 – Anti-Thyroid Peroxidase Human ELISA Kit**

## Instructions for Use

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

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

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## 1. BACKGROUND

Abcam's anti-Thyroid Peroxidase Human *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative determination of autoantibodies to thyroid peroxidase in Human serum or plasma (citrate).

A 96-well plate has been precoated with recombinant thyroid peroxidase 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 thyroid peroxidase -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 thyroid peroxidase sample captured in plate.

Thyroid peroxidase is present at the apical plasma membrane of Human thyroid cells catalyzes the first step in thyroid hormone synthesis. Thyroid peroxidase oxidizes iodide-anions and iodinates some tyrosyl residues of thyroglobulin (TG), leading to the formation of monoiodotyrosine (MIT) and diiodotyrosine (DIT). Coupling of MIT and DIT yields tri-iodotyronine (T<sub>3</sub>) and tetra-iodotyronine (T<sub>4</sub>).

Thyroid peroxidase (previously known as thyroid microsomal antigen) is a frequent epitope of autoantibodies in autoimmune thyroid disease. Thyroid peroxidase autoantibodies (thyroid peroxidaseAb) are detectable in over 90 % of patients with Hashimoto's thyroiditis and over 70 % of those with Graves' disease (autoimmune hyperthyroidism), irrespective of the functional state of the thyroid gland.

Postpartum thyroiditis is a special form of autoimmune thyroiditis observed following pregnancy and may involve temporary hyperthyroidism or hypothyroidism. It affects about 5 % of all women within a year after giving birth. The risks are higher for women with autoimmune disorders (such as type 1 diabetes), positive anti-thyroid antibodies or a history of previous thyroid dysfunction.

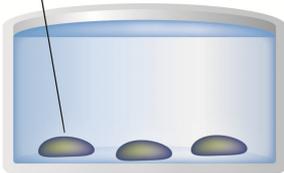
Moderately elevated thyroid peroxidaseAb values are sometimes found in patients without other evidence of thyroid disease, especially in the

elderly. The clinical significance of low levels of thyroid peroxidaseAb in euthyroid subjects is still unknown. Whether these individuals should be considered normal remains in question until long-term followup studies show that they do not have an increased risk for developing thyroid dysfunction.

Autoantigen	Disease	Symptoms
Thyroid Peroxidase	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)

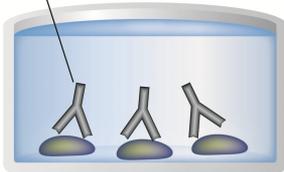
## 2. ASSAY SUMMARY

### Capture Antigens



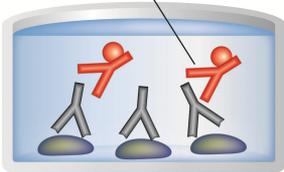
Prepare all reagents, samples and controls as instructed.

### Sample



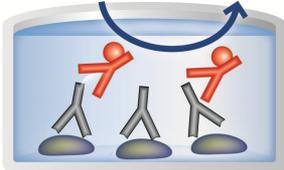
Add samples and controls to wells used. Incubate at 37°C.

### Labeled HRP-Conjugate



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

### Substrate Colored Product



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.**

Individual prepared components should also be stored at 2-8°C

## 5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Thyroid Peroxidase 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
Thyroid Peroxidase anti-IgG HRP Conjugate**	20 mL	2-8°C
TMB Substrate Solution	15 mL	2-8°C
Thyroid Peroxidase Negative control***	2 mL	2-8°C
Thyroid Peroxidase Positive control***	2 mL	2-8°C
Thyroid Peroxidase Standard A – 0 U/mL***	2 mL	2-8°C
Thyroid Peroxidase Standard B – 5 U/mL***	2 mL	2-8°C
Thyroid Peroxidase Standard C – 10U/mL***	2 mL	2-8°C
Thyroid Peroxidase Standard D – 20 U/mL***	2 mL	2-8°C
Thyroid Peroxidase Standard E – 80 U/mL***	2 mL	2-8°C
Thyroid Peroxidase Standard F – 320 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  $\mu$ L sample to 990  $\mu$ L IgG Sample Diluent to obtain a 1:100 dilution. Mix gently and thoroughly.
- For samples with expected anti-Thyroid Peroxidase concentrations greater than Standard F (320 U/mL) a second 1:1 dilution of this 1:100 diluted sample should be performed; e. g. 100  $\mu$ L of first sample dilution + 100  $\mu$ L 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  $\mu$ L to 350  $\mu$ L to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**
- **All controls (thyroid peroxidase positive and thyroid peroxidase 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  $\mu$ L 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  $\mu$ L of 1X Washing Solution. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. 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.

## ASSAY PROCEDURE

- 13.6. Add 100  $\mu$ L thyroid peroxidase 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  $\mu$ L TMB Substrate Solution into all wells
  - 13.10. Incubate for exactly 15 minutes at room temperature in the dark.
  - 13.11. Add 100  $\mu$ L Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
- 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 < **25 U/mL**
- **Positive Control:** Absorbance value > **40 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

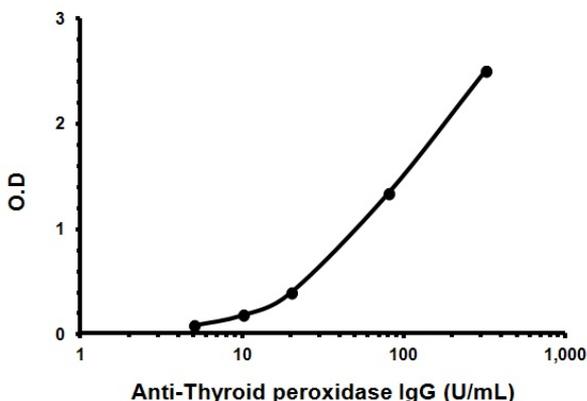
Normal value ranges for this ELISA should be established by each researcher.

The following values should be considered as a guideline only:

Normal	< 30 U/mL
Inconclusive (Grey zone):	30 - 35 U/mL
Non reactive:	> 35 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 –**

Standard	Intra-Assay			Inter-Assay	
	Positive serum	Positive serum	Negative Serum	Positive serum	Positive serum
n=	20	20	20	12	12
Mean (OD)	1.334	1.265	0.209		
Mean (U/mL)				113.3	176.0
%CV	3.8	4.8	6.0	7.1	5.7

## **17. ASSAY ANALYTICAL SPECS**

### **SPECIFICITY -**

The specificity is > 97 % and is defined as the probability of the assay scoring negative in the absence of the specific analyte.

### **SENSITIVITY -**

The sensitivity is > 97 % 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 too 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)

## RESOURCES

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
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

# RESOURCES





**UK, EU and ROW**

Email: [technical@abcam.com](mailto:technical@abcam.com) | Tel: +44-(0)1223-696000

**Austria**

Email: [wissenschaftlicherdienst@abcam.com](mailto:wissenschaftlicherdienst@abcam.com) | Tel: 019-288-259

**France**

Email: [supportscientifique@abcam.com](mailto:supportscientifique@abcam.com) | Tel: 01-46-94-62-96

**Germany**

Email: [wissenschaftlicherdienst@abcam.com](mailto:wissenschaftlicherdienst@abcam.com) | Tel: 030-896-779-154

**Spain**

Email: [soportecientifico@abcam.com](mailto:soportecientifico@abcam.com) | Tel: 911-146-554

**Switzerland**

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