

# **TECO® TSH-Receptor**

## **TECO® TSH Receptor Autoantibody 2<sup>nd</sup> Generation ELISA KIT**

Instructions for Use  
English



Catalogue No. TE1010

*always your partner*

# Symbol Description



Kit Instructions



Lot Number



Expiry Date



In Vitro Diagnostic



CE Declaration of Conformity  
H-CH/CA01/IVD/18673



Storage Temperature



Manufacturer



Caution: read instructions



TE 1010



Caution: caustic



Intended use



Tests

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# TECO® TSH Receptor Autoantibody 2<sup>nd</sup> Generation ELISA Kit

## **CONT** Reagents and Materials Supplied:

Symbol	Description	Format
<b>1</b>	<b>TSH Receptor Coated Microtiter Plate</b> 12 strips of 8 wells (96 breakable wells in total), in a frame, Ready to use	1 plate
<b>2</b>	<b>Start Buffer</b> Ready to use, yellow solution	1 x 10 ml
<b>A</b>	<b>Standard A</b> 0 U/l (IgG, human), ready to use, blue cap	1 x 1,0 ml
<b>B</b>	<b>Standard B</b> 1 U/l (IgG, human), ready to use	1 x 1,0 ml
<b>C</b>	<b>Standard C</b> 2 U/l (IgG, human), ready to use	1 x 1,0 ml
<b>D</b>	<b>Standard D</b> 8 U/l (IgG, human), ready to use	1 x 1,0 ml
<b>E</b>	<b>Standard E</b> 40 U/l (IgG, human), ready to use	1 x 1,0 ml
<b>L</b>	<b>Control 1</b> (IgG, human) ready to use, yellow cap, Range see data sheet	1 x 1,0 ml
<b>H</b>	<b>Control 2</b> (IgG, human) ready to use, yellow cap, Range see data sheet	1 x 1,0 ml
<b>3</b>	<b>TSH Biotin</b> Biotin-labeled thyrotropin, lyophilised	3 x 4,5 ml
<b>4</b>	<b>TSH Biotin Reconstitution Buffer</b> Ready to use, red solution	1 x 15 ml
<b>5</b>	<b>Enzyme-HRP Conjugate (SA-HRP)</b> Peroxidase-labeled streptavidin, 20 times concentrated	1 x 0,75 ml
<b>6</b>	<b>Diluent for SA-HRP Conjugate</b> Ready to use	1 x 15 ml
<b>7</b>	<b>TMB Substrate</b> TMB/H <sub>2</sub> O <sub>2</sub> , ready to use	1 x 15 ml
<b>8</b>	<b>Wash Solution</b> 10 times concentrated	1 x 100 ml
<b>9</b>	<b>Stop Solution – 0,5 M H<sub>2</sub>SO<sub>4</sub></b> 0,5 M sulfuric acid, ready to use	1 x 10 ml
	<b>Kit instruction</b>	1 x



## **Storage**

Store kit at 2–8 °C. Do not freeze. Store unused reagents at 2–8 °C.

## **Instructions for Use**

The TECO® TSH Receptor Autoantibody 2<sup>nd</sup> Generation Kit is an ELISA intended for the quantitative determination of TSH receptor autoantibodies in human serum (Rees Smith et al., 2004). Hyperthyroidism in Graves' disease is due to the presence of autoantibodies to the TSH receptor. Measurement of these autoantibodies can be useful in disease diagnosis and management.

## **Clinical Use**

Primary thyroid disease often develops due to an autoimmune process which leads to the formation of antibodies to one or more of the three thyroid autoantigenes, namely thyroid peroxidase (TPO), thyroglobin (TG), and thyrotropin (TSH) receptor.

Auto antibodies directed against the TSH receptor may cause hyperthyroidism (Graves' disease). This disease affects around 2% of the female and 0,2% of the male population.

TSH receptor autoantibodies are heterogeneous with respect to their biological mode of action. Antibodies can be present which have a stimulating or blocking effect on the TSH receptor: i. e., antibodies that stimulate the growth of the thyroid and antibodies which inhibit the binding of TSH to the TSH receptor (TBII, TSH-binding inhibitory immunoglobulins).

The biological activity of a patient's autoantibodies to the TSH receptor can change, e. g., there may occur a shift from blocking to stimulating activity - the reverse is rare. The measurement of TSH receptor autoantibodies is mainly used in the assessment of patients with suspected Graves' disease. Graves' disease is an autoimmune disease that is characterized by thyroid overactivity (hyperthyroidism). Further cardinal symptoms are goiter, exophthalmus, and tachycardia (Merseburg trias).

Determinations of TSH receptor autoantibodies are clinically used to confirm the diagnosis of Graves' disease and to differentiate the disease from disseminated autonomy of the thyroid. Around 98% of the patients diagnosed with Graves' disease react positively in the TSH receptor antibody assay.

The determination of TSH receptor autoantibodies in the course of Graves' disease has predictive value and serves as an important tool in therapy monitoring. High levels of TSH receptor autoantibodies after longer-term thyrostatic therapy in patients with Graves' disease indicate an increased risk of relapse.

Furthermore, increased concentrations of TSH receptor autoantibodies in the third trimester of pregnancy in women with Graves' disease are indicative of hyperthyroidism in the fetus. In pregnant women diagnosed with Hashimoto thyroiditis and myxoedema blocking TSH receptor autoantibodies may pass through the placenta and cause temporary neonatal hypothyroidism.

The measurement of TSH receptor autoantibodies may also be helpful in ophthalmology as many patients, showing symptoms of Graves' disease, first consult an ophthalmologist.

## References

- 1 Rees Smith B et al. (2004)  
**A new assay for thyrotropin-receptor-auto-antibodies.**  
Thyroid 14: 830-835.
- 2 Kamij K (2003)  
**TSH receptor-antibody measurement in patients with various thyrotoxicosis and Hashimoto's thyroiditis: a comparison of two-step assays, coated plate ELISA using porcine TSH receptor and coated tube radioassay using human recombinant TSH receptor.**  
Endocrine Journal 50: 113-116.
- 3 Rees Smith B (2001)  
**Thyroid autoantibodies.**  
The Scandinavian Journal of Clinical & Laboratory Investigation Supplement 61: 45-52.
- 4 Orgiazzi J (2000)  
**Anti-TSH receptor-antibodies in clinical practice.**  
Endocrinology and Metabolism Clinics of North America 29: 339-355.
- 5 Gupta MK (2000)  
**Thyrotropin-receptor-antibodies in thyroid diseases: advances in detection techniques and clinical applications.**  
Clinica Chimica Acta 293: 1-29.
- 6 Bolton J, Sanders J, Oda Y, Chapman C, Konno R, Furmaniak J, Rees Smith B (1999)  
**Measurement of thyroid-stimulating hormone receptor autoantibodies by ELISA.**  
Clinical Chemistry 45: 2285-2287.

## Assay Principle

This TECO® TSH Receptor Autoantibody 2<sup>nd</sup> Generation ELISA Kit kit is intended for the quantitative in vitro determination of human thyrotropin receptor autoantibodies in human serum. **The microwell plate contains (12 strips of 8 wells each) 96 wells coated with porcine TSH receptor.**

A monoclonal antibody specific for the TSH receptor is used to bind the receptor to the micro-wells. Human TSH receptor autoantibodies show the same reactivity to porcine and human TSH receptor.

In a first analytical step, patient sera are incubated. In positive samples specific antibodies bind to the TSH receptors. The bound antibodies inhibit the binding of biotin-labeled TSH, which is added into the wells in a second incubation step. The amount of bound biotin is then determined in a third incubation step by addition of enzyme-labeled streptavidin (enzyme conjugate) that catalyzes a colour reaction. The intensity of the colour reaction is reciprocally proportional to the concentration of TSH receptor antibodies in the test sample.

## Materials Required and not Supplied

- Pipettes capable of dispensing 50 µl, 100 µl and 350 µl
- Graduated cylinders for reconstituting or diluting reagents
- Automatic washer or multi-channel pipette
- Aqua dest
- Vortex mixer
- ELISA plate reader suitable for 96 well formats and capable of measuring at 450 nm, and 590-650 nm for reference.
- ELISA plate shaker (500 rpm) (orbital shaker)
- Software package for data reduction and analysis

## Warnings and Precautions

This kit is intended for in vitro use by professional persons only.

### **Follow the instructions carefully.**

Observe expiration dates stated on the labels and the specified stability for reconstituted reagents. Refer to "Materials Safety Data Sheet" for more detailed safety information.

Material of human origin used in the preparation of this kit has been tested and found non-reactive for HIV-1 and HIV-2 as well as for HCV antibodies and HbsAg but should, nonetheless, be handled as potentially infectious.

Material of animal origin used in the preparation of this kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.

TECOmedical AG is not liable for loss or harm caused by non-observance of the Kit instruction.

1. For in vitro diagnostic use.
2. Treat all specimen samples as potentially biohazardous material. Follow general precautions when handling contents of this kit and any patient samples.
3. Disposal of containers and unused contents should be done in accordance with federal and local regulatory requirements.
4. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
5. Store assay reagents as indicated.
6. Do not use coated strips if pouch is punctured.
7. Test each sample in duplicate.
8. Use of multichannel pipettes or repeat pipettors is recommended to ensure the timely delivery of liquids.
9. 0,5 M sulfuric acid is caustic and can cause severe burns. Do not ingest. Avoid contact with skin, eyes, or clothing. Should there be any contact, wash with water. If ingested, call a physician.
10. Sodium azide is used as a preservative. Incidental contact or ingestion of buffer solutions containing sodium azide can cause irritation of skin, eyes or mouth. Sodium azide may react with lead, copper or brass plumbing to form explosive metal azides. On disposal, flush with a large amount of water to prevent azide build-up.

## Preparation of Reagents

- 1 TSH Receptor Coated Microtiter Plate**  
12 breakapart strips of 8 wells (96 wells) in a frame and sealed in a foil bag. Fit strip wells firmly into the frame. After opening, immediately return any unused wells to the original foil package and seal. Store at 2–8 °C until expiration date.
- 2 Start Buffer**  
10 ml, ready to use.  
Store at 2–8 °C until expiration date.
- A  
till  
E Standards**  
5 x 1,0 ml, ready to use. 0 U/l, 1 U/l, 2 U/l, 8 U/l and 40 U/l. Units are WHO Standards 90/672. Store at 2–8 °C until expiration date.
- L Control 1**  
1,0 ml, ready to use. Range as indicated on data sheet.  
Store at 2–8 °C until expiration date.
- H Control 2**  
1,0 ml, ready to use. Range as indicated on data sheet.  
Store at 2–8 °C until expiration date.
- 3 TSH Biotin**  
3 x 4,5 ml lyophilised. Reconstitute each vial with 4,5 ml TSH Biotin Reconstitution Buffer . When more than one vial is to be used, pool the vials after reconstitution and mix gently before use. Store at 2–8 °C for up to 12 weeks after reconstitution.
- 4 TSH Biotin Reconstitution Buffer**  
15 ml, ready to use.  
Store at 2–8 °C until expiration date.
- 5 Enzyme Conjugate (SA-HRP)**  
1 x 0,75 ml, concentrated. Dilute 1:20 with Diluent for SA-HRP . For example, 0,6 ml + 11,4 ml . Store at 2–8 °C for up to 12 weeks after dilution.
- 6 Diluent for SA-HRP**  
15 ml, ready to use.  
Store at 2–8 °C until expiration date.

**7 TMB Substrate**

15 ml, ready to use.  
Store at 2–8 °C until expiration date.

**8 Concentrated Wash Solution**

100 ml, concentrated. Dilute to 1 liter with distilled water before use.  
Store at 2–8 °C until expiration date.

**9 Stop Solution – 0,5 M H<sub>2</sub>SO<sub>4</sub>**

10 ml, ready to use.  
Store at 2–8 °C until expiration date.

## Preparation and Stability of Serum Samples

### Sample Type

Human serum.

### Stability

Serum samples to be tested can be kept up to 14 days at 2–8 °C. Grossly haemolytic or lipemic serum samples should not be used. **Do not use plasma samples.**

Sera to be tested should be assayed soon after separation or stored, preferably in aliquots, at or below -20 °C. 250 µl are sufficient for one assay (duplicates of 100 µl determinations are recommended). Repeated freezing and thawing should be avoided. When required, thaw test sera at room temperature and mix gently to ensure homogeneity. Incorrect storage of serum samples can lead to loss of TSH receptor autoantibody activity.

## Assay Procedure

Allow all reagents to stand at room temperature (20–25 °C) for at least 30 minutes.

1. Prepare the frame and the required number of coated strips . Allocate the wells of the Microtiter plate for Standard, Controls and Samples.
2. Pipette 50 µl of Start Buffer into each well.
3. Pipette 100 µl of patient sera, Standards till , and Controls and into respective wells.
4. Cover the frame and shake the wells for 2 hours at room temperature (20–25 °C) on an ELISA plate shaker (500 rpm).
5. After incubation, aspirate the wells by using a plate washer or manually decant by inverting the plate. Wash the wells 2 x with 350 ml diluted washing buffer. After the last wash cycle tap the inverted wells gently on a dry absorbent surface to remove excess wash solution.
6. Pipette 100 µl of reconstituted TSH Biotin into each well. Avoid splashing the material out of the wells during addition.
7. Cover the plate and incubate at room temperature for 25–30 minutes without shaking.
8. Wash 3 x as described in step 5.
9. Pipette 100 µl of diluted Enzyme Conjugate (SA-HRP) into the wells and incubate at room temperature for 25–30 minutes without shaking.
10. Wash 3 x as described in step 5.
11. Incubate 100 µl TMB into each well and incubate in the dark at room temperature for 25–30 minutes without shaking.
12. Pipette 50 µl Stop Solution into each well and shake the plate for approximately 5 seconds on a plate shaker and read the absorbance within 15 minutes.
13. Read the absorbance of each well at 450 nm using an ELISA plate reader (Reference filter: 590–650 nm).

**Protocols for the different automatic ELISA systems are available.**

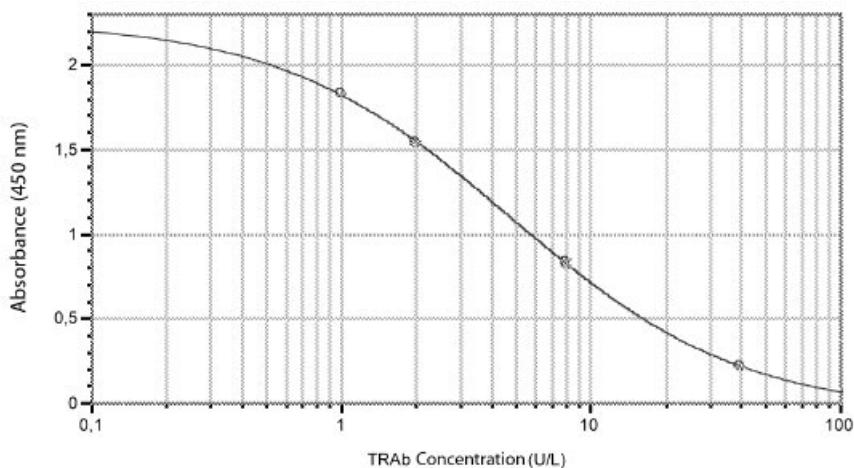
## Result Analysis

A standard curve can be established by plotting standard concentration on the x-axis (log scale) against the absorbance of the standards on the y-axis (linear scale). The TSH receptor autoantibody concentrations in patient sera can then be read off the standard curve. A 4-parameter curve fit should be used for automatic data reduction.

### Typical Results 450 nm

(Example only. Not for use in calculation of actual results)

Sample	Absorbance at 450 nm	U/l
A	2,248	0
B	1,829	1
C	1,535	2
D	0,827	8
E	0,211	40
L-Control 1	2,01	<1
H-Control 2	1,180	4,1



$$y = \left( \frac{A - D}{1 + (x/C)^B} \right) + D$$

○ STD#1 (Standards: Concentration vs MeanValue)

A      B      C      D      R<sup>2</sup>  
 2,25    0,936    4,857    -0,071    1

For each assay, the results of the controls must be within the target range indicated for every lot. The QC protocol with target ranges is provided with the kit. If control values are not within the limits of the target range, the assay results should be considered questionable and the samples should be tested again.

## Reference Values

### Recommended Range of Reference Values

Cut-off	U/l
Negative	< 1,0
Grey zone	1,0–2,0
Positive	> 2,0

## Clinical Evaluation

### Clinical Specificity

154 samples from healthy blood donors were assayed using the TSH Receptor Autoantibody ELISA kit. 99% were identified as being negative for TSH receptor autoantibodies.

### Clinical Sensitivity

50 samples from patients diagnosed with Graves' disease were assayed using the TSH Receptor Autoantibody ELISA kit. 98% were identified as being positive for TSH receptor autoantibodies. 2% were identified as being within the grey zone.

### Clinical Accuracy

Analysis of samples from patients with autoimmune diseases other than Graves' disease indicated no interference from autoantibodies to thyroglobulin, thyroid peroxidase, glutamic acid decarboxylase, 21-hydroxylase, acetylcholine receptor, dsDNA or rheumatoid factors.

### Interference

No interference was observed when samples were spiked with the following materials: haemoglobin up to 5 mg/ml, bilirubin up to 0,2 mg/ml, human LH up to 10 U/ml, hCG up to 160 U/ml, human FSH up to 70 U/ml and human TSH up to 30 U/l.

## Performance

### Standard

This test is standardised against the 1<sup>st</sup> International Standard for Thyroid stimulating antibodies (WHO, 1995, Standard 90/672, National Institute for Biological Standards and Control, Hertfordshire, England).

### Precision

#### (Inter assay)

Sample	Mean value U/l	Between %CV n=6
1	1,4	7,6
2	1,9	4,0
3	2,7	5,0
4	3,4	8,6
5	19,7	6,0

#### (Intra assay)

Sample	Mean value U/l	Within %CV n=5
1	3,26	4,3
2	5,50	0,8
3	7,77	1,6
4	17,82	5,9

### Detection Limit

The kit negative control was assayed 16 times and the mean and standard deviation calculated. The lower detection limit at +2 standard deviations was 0,25 U/l.

## Dilution Test

Sample	Dilution Factor	Expected U/l	Observed U/l	Recovery (%)
Serum 1	1	41,30	41,30	100,00
	2	20,60	25,90	125,40
	4	10,30	12,20	117,80
	8	5,20	5,80	112,50
	16	2,60	2,90	110,90
Serum 2	1	31,60	31,60	100,00
	2	15,80	17,10	108,40
	4	7,90	8,40	106,30
	8	4,00	4,30	109,20
	16	2,00	2,50	126,50
Serum 3	1	41,00	41,00	100,00
	2	20,50	22,40	109,30
	4	10,30	10,40	101,20
	8	5,10	4,70	91,20
	16	2,60	2,30	89,50
Serum 4	1	18,70	18,70	100,00
	2	9,40	9,20	98,00
	4	4,70	4,50	96,40
	8	2,30	2,50	107,20
	16	1,20	1,60	133,50
Serum 5	1	28,70	28,70	100,00
	2	14,30	16,00	111,70
	4	7,20	7,70	108,10
	8	3,60	3,90	109,30
	16	1,80	1,90	106,40
Serum 6	1	9,00	9,00	100,00
	2	4,50	4,50	100,80
	4	2,20	2,40	107,50
	8	1,10	1,20	110,60
	16	0,60	0,70	130,00

## Recovery

Four negative serum samples have been spiked with 8 different concentrations of TSH receptor autoantibodies (2-30 U/L)

Sample	Mean %-recovery
A	121
B	109
C	106
D	117

## Remark

The data quoted in this instruction should be used for guidance only. It is recommended that each laboratory includes its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological ranges for TSH receptor autoantibody levels according to GLP guidelines.

# TECO® TSH Receptor Autoantibody 2<sup>nd</sup> Generation

## Assay Procedure – Quick Guide

- Bring samples and reagents to room temperature.
- Reconstitute each vial of TSH-Biotin **3** with 4,5 ml Reconstitution Buffer **4**.
- Dilute Wash Buffer concentrate **8** up to 1 l of Aqua dest.
- Dilute SA-HRP **5** 1:20 with Diluent **6** e. g. 0,6 + 11,4 ml.

Prepare the required number of Assay Strips

Pipette **50 µl** Start Buffer **2** into each well

Pipette **100 µl** Standards **A** till **E**, Controls **L** and **H** and Samples

Cover the plate and incubate **120 min** at 20–25 °C on a rotator 500 rpm

Aspirate, wash **2 x** with at least **350 µl** Washing Buffer, aspirate and tap the inverted wells gently on a clean dry absorbent surface

Pipette **100 µl** reconstituted TSH Biotin into each well

Cover the plate and incubate **25–30 min** at 20–25 °C

Aspirate, wash **3 x** with at least **350 µl** Washing Buffer, aspirate and tap the inverted wells gently on a clean dry absorbent surface

Pipette **100 µl** diluted SA-HRP into each well

Cover the plate and incubate **25–30 min** at 20–25 °C

Aspirate, wash **3 x** with at least **350 µl** Washing Buffer, aspirate and tap the inverted wells gently on a clean dry absorbent surface

Pipette **100 µl** TMB **7** into each well

Cover plate and incubate **25–30 min** at 20–25 °C

Pipette **50 µl** Stop Solution **9** into each well and shake 5 seconds

Read optical Density at **450 nm**.

Reference measurement should be performed at 590–650 nm

Analyze the assay results (Quantification software, 4-parameter fit:

$$y = (A-D)/(1+(x/C)^B)+D$$



Please read Kit instruction before using the Quick Guide