

ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	#1200	Human Albumin (Urinary)
#1750	Human IgG (total)	#1760	Human IgM
#1800	Human IgE	#1810	Human Ferritin
#1210	Human Transferrin (Tf)	#0020	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		
#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	#1830	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	#1510	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		
#0030	Human Insulin	#0040	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#0410	HCG-free beta
#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	#1110	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	#1700	Human T3 (total)
#1850	Human Cortisol	#1860	Human Progesterone
#1865	Human Pregnenolone	#1875	Human Aldosterone
#1880	Human Testosterone	#1885	Human free Testosterone
#1910	Human Androstenedione	#1920	Human Estradiol
#1925	Human Estrone	#1940	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		
#3000	Human Rheumatoid Factors IgM (RF)		
#3100	Human anti-dsDNA		
#3200	Anti-Nuclear Antibodies (ANA)		

Instruction Manual No. M-610

Human TSH Receptor Autoantibody (TSHR ab) ELISA KIT Cat. # 610, 96 Tests

For Quantitative Determination of thyrotropin receptor (TSHR) autoantibodies in human serum, plasma or other biological fluids

For In Vitro Research Use Only



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Human TSH Receptor Autoantibody (TSHR ab) ELISA KIT Cat. #. 610

Kit Contents: (reagents for 96 tests)

Components	Qty
TSH receptor coated microwell strip plate (8x12 or 96 wells) #6 1 1	1Plate
TSHR Ab Standard (Powder) # 612; to be reconstituted	2 vials
Biotinylated antibody (100X), 130 ul; # 6 1 3	1 vial
Enzyme Conjugate (100X), 130 ul; # EC-6 1 0	1 vial
Enzyme Diluent, 12 ml# 614	3 vials
Antibody Diluent, 12 ml #; #615	1 bottle
Standard Diluent, 15 ml #616	1 vial
Sample Diluent, 15 ml # 617	1 bottle
Wash Buffer Conc (25X). 20 ml, Dilute with 950 ml of distilled water, # WB-610	1 bottle
Color Reagent A, 10 ml # 618A	
Color Reagent B, 1.5 ml # 618B	
Color Reagent C, 10 ml # 618C	
Stop solution, 10 ml,# S T - 6 1 0	1 bottle
Complete Instruction Manual, M - 6 1 0	1

Intended Use

TSH receptor is Double Antibody Sandwich ELISA for the detection of TSHR antibody (Ab/TRAb). It is intended for the measurement of thyrotropin receptor autoantibodies in human serum. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

INTRODUCTION

The secretion of TSH from the anterior pituitary is controlled by thyrotropin releasing hormone (TrRH) produced by the hypothalamus. Thyroid disorders are the most prevalent of all autoimmune diseases. Thyroid autoimmune diseases are associated with the occurrence of differentiated autoantibodies and are thought to be related to a genetic pre-disposition. These autoantibodies are directed against membrane-located and/or extracellular antigens of the thyroid cells. The thyrotropin receptor (TSH receptor) is the antigen for TSH receptor antibodies (TRAbs). The TSH receptor signals through Gs to elevate intracellular cAMP in the thyroid gland, which regulates iodide uptake, and transcription of thyroglobulin (Tg), thyroid peroxidase (TPO), and sodium-iodide symporter. TRAbs are grouped depending on their effects on receptor signaling; activating antibodies (associated with hyperthyroidism), blocking antibodies (associated with thyroiditis) and neutral antibodies (no effect on receptor). Activating and blocking antibodies mostly bind to conformational epitopes, whereas neutral antibodies bind to linear epitopes

Autoimmunity to the TSH receptor causes hyperthyroidism (Graves disease) or hypothyroidism (Hashimoto thyroiditis) when the auto function as agonists or antagonists, respectively. The production of antibodies in Graves' disease is thought to arise by activation of CD4+ T-cells, followed by B-cell recruitment into the thyroid. These B-cells produce antibodies specific to the thyroid antigens. In Hashimoto's thyroiditis, activated CD4+ T-cells produce interferon- γ , causing the thyroid cells to display MHC class II

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Detection range: 100 ng/ml- 1.56 ng/ml

2. PRECISION

Intra-assay precision: $\leq 8\%$

Inter-assay precision: $\leq 12\%$

Recovery: 70 - 110 percent

Interference: No interference was observed when sample were spiked with the following materials:

Haemoglobin at 5 mg/ml; bilirubin at 0.2 mg/ml; intralipid at 30 mg/ml; human LH up to 10 U/ml; hCG up to 160 U/L; human FSH up to 70 U/mL; human TSH up to 30 U/L.. The data quoted above should be used for guidance only. Each laboratory should establish its own normal & pathological reference ranges for TrAb levels.

REAGENTS PREPARATION FOR THE ASSAY:

1. **Before use dilute wash buffer (25X): Dilute 1:25 in water or dilute the entire bottle in 1-Liter of distilled water.** Diluted buffer can be stored at 4oC for several weeks.
2. Reconstitute lyophilized **Human TSHR Ab standard** by adding **1.0 ml** of **standard diluent** into lyophilized standard and gently mix it at room temp for 30 min. Do not vortex. TSHR antibody stock concentration is 100 ng/ml (std #1). It should be used immediately or the same day. Do not store this stock beyond the assay date or re-use. There are 2 vials of lyophilized standards provided and the 2nd vial to be used if needed. Need 100 ul x2 of the standards for each test in duplicate.
3. Prepare **addition standards** #2-8 of 50, 25, 12.5, 6.25, 3.12,1.56 ng/ml by performing 2-fold serial dilutions using **standard diluent as follows.**

Std #	Volume	Standard diluent	Final Conc (ng/ml)
1	Prepared in Step 2	1 ml	100
2	200 ul of Std 1	200 ul	50
3	200 ul of Std 2	200 ul	25
4	200 ul of Std 3	200 ul	12.5
5	200 ul of Std 4	200 ul	6.25
6	200 ul of Std 5	200 ul	3.12
7	200 ul of Std 6	200 ul	1.56

4. **Biotinylated human TSHR Ab antibody (100X):** Dilute 1:100 with **antibody diluent** (10 ul stock in 990 ul of antibody diluent or 100 ul stock in 9.9 ml of diluent). The preparation should be done 30 min in advance. Prepare as needed. Prepare 1 ml for each 8-well strip or 10 ml for the entire plate. Do not store the diluted antibody beyond the assay date.
5. **Enzyme-conjugate (100x):** Dilute 1:100 with **enzyme-conjugate diluent** (10 ul stock in 990 ul of antibody diluent or 100 ul stock in 9.9 ml of diluent). The preparation should be done 30 min in advance. Prepare as needed. Prepare 1 ml for each 8-well strip or 10 ml for the entire plate. Do not store the diluted antibody beyond the assay date.
6. Prepare **TMB working Substrate Reagent** at least 30-60 min before use. Mix 900 ul Color Reagent A and 100 ul of Color Reagent B (9:1 ratio). Prepare 1 ml for each 8-well strip or 10 ml for the entire plate.

STORAGE AND STABILITY:

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping. Standards are stable for two month at 2-8°C. The unused portions of the standards can be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE:

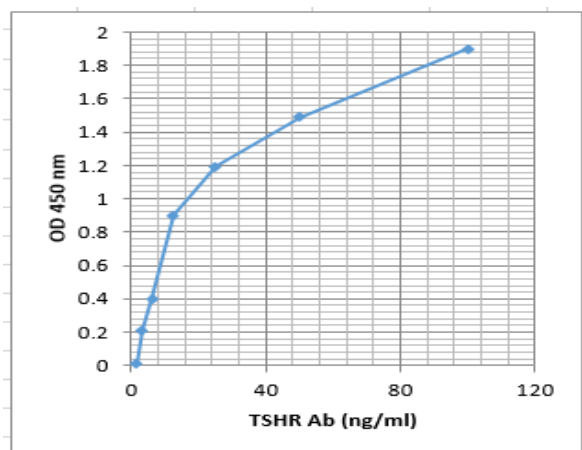
(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE (20oC-25 oC) for at least 30 minutes BEFORE USE).

1. Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Label or mark the microtiter well strips to be used on the plate.
2. Add 100 ul of sample diluent into 2 wells as blank (zero standard). Pipette **100 µL** of diluted **standards (1-7) and diluted samples** into respective wells (start with the 100 ng/ml std and descend down the plate to the low control and then test sera), leaving the last well blank. Mix gently for 5-10 seconds.
3. Cover the plate and incubate for **90 mins at 37 °C.**
4. After incubation, aspirate the wells and **Wash the wells 3 times with 1X wash solution.** After the last wash, Tap the inverted wells gently on a clean, dry, absorbent surface to remove excess wash solution.
5. Add **100 µL** of 1X **Biotinylated TSH** into each well (except blank). Mix gently for 5-10 seconds.
6. Cover the plate, and **incubate for 60 mins at 37 °C.**
7. **Repeat wash step 4.**
8. Add **100 µL** of 1X **Enzyme Conjugate (Streptavidin-HRP)** into each well (except blank) and incubate at room temperature for **30 mins at 37 °C.**
9. After incubation, **Wash the wells 5 times as in step 4.** After the last wash, Tap the inverted wells gently on a clean, dry, absorbent surface to remove excess wash solution.
10. Add **100 µL** of **working substrate into each well** (including blank). Mix gently for 5-10 seconds and incubate in the dark at **at 37 °C for 30 minutes.** Blue color develops in positive wells.
11. Add **50 µL stop solution** to each well (including blank) and mix gently for 5-10 seconds. Or shake the plate for approximately 5 seconds on a plate shaker. Blue color turns yellow.
12. Read the absorbance of each well at **450 nm** using an ELISA plate reader within 10-15 mins. A reference filter of 630nm may be used.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (ng/ml)	Mean A _{450nm}	Net A _{450nm}
A1, A2	Blanks	0.100	-
B1, B2	Std. A (1.56)	0.21	0.11
C1, C2	Std. B (3.12)	0.31	0.21
D1, D2	Std. C (6.25)	0.51	0.4
E1, E2	Std. D (12.5)	1.091	0.9
F1, F2	Std. E (25)	1.29	1.191
G1, G2	Std. F (50)	1.59	1.49
H1, H2	Std. G (100)	1.9	1.9

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



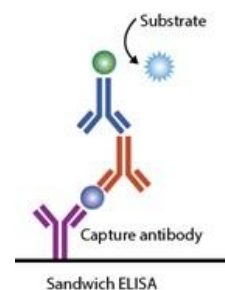
*7-ADI-ELISA

A calibration curve can be established by plotting calibrator concentration on the x-axis against the absorbance of the calibrators on the y-axis (linear scale). The TRAb concentrations in sera can then be read off the calibration curve. Other data reduction systems can be used.

molecules. The presence of anti-thyroid antibodies is also associated with an increased risk of unexplained sub fertility.

ADI's Anti-Thyroid Stimulating Hormone receptor (TSHR) antibody ELISA Kit is used for the quantitative measurement of thyrotropin receptor autoantibodies in human serum. Kit for the measurement of thyroid stimulating receptor in human samples is also available

PRINCIPLE OF THE TEST



This experiment use double-sandwich elisa technique. The pre-coated antibody is human TSHR Ab monoclonal antibody and the detecting antibody is polyclonal antibody with biotin labeled. Samples and biotin labeling antibody are added into ELISA plate wells and washed to remove unbound antibodies. Bound antibodies are then detected using the Avidin-peroxidase conjugates. After another washing step; TMB substrate is added and blue color develops in positive wells. Stop solution converts blue to yellow and A450nm is measure using an ELISA reader. TSHR antibody concentrations are determined relative to the standards provided in the kit.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (50-200 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International TSHR Ab ELISA kit is intended for *in vitro research* use only. The Control and Standards have been prepared from human sera shown to be negative for HbsAg, HCV and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera, and waste solutions should be handled with appropriate precautions and disposed properly.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H₂SO₄ (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed , these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum. Samples can be tested undiluted or diluted (1:10 or more to bring them within the testing range.