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 Pregnolone	**#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-600

Human Thyroid-Stimulating Hormone (TSH)

ELISA KIT Cat. No. 600

For Quantitative Determination of TSH In Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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Web Site: www.4adi.com

Human Thyroid-Stimulating Hormone (TSH)
ELISA KIT Cat. No. 600
For Quantitative Determination of Human TSH In Serum

Kit Contents: (reagents for 96 tests)

Components	
Anti-hTSH coated microwell **strip plate**(96 wells) #6 0 1	**1 Plate**
TSH **Std. A**, 0.7 ml, 0 uIU/mL, #6 0 2 A	1 vial
TSH **Std. B**, 0.70 ml, 0.5 uIU/mL, #6 0 2 B	1 vial
TSH **Std. C**, 0.70 ml, 2.5 uIU/mL, #6 0 2 C	1 vial
TSH **Std. D**, 0.70 ml, 5 uIU/mL, # 6 0 2 D	1 vial
TSH **Std. E**, 0.70 ml, 10 uIU/mL, #6 0 2 E	1 vial
TSH **Std. F**, 0.70 ml, 20 uIU/mL, #6 0 2 F	1 vial
TSH **Std. G**, 0.70 ml, 40 uIU/mL, #6 0 2 G	1 vial
Anti-TSH-Enzyme **Conjugate**, 12 ml, #6 0 3	1 bottle
HRP Substrate **Solution** , 12 ml, # T M B - 6 0 0	1 bottle
Wash Buffer (20X) 25 ml, Dilute 1:20 with distilled water, # W - 2 0	1 bottle
Stop solution, 12 ml,# S T - 6 0 0	1 bottle
Complete Instruction Manual, M 6 0 0	1

INTRODUCTION

The secretion of TSH from the anterior pituitary is controlled by thyrotropin releasing hormone (TRH) produced by the hypothalamus. TSH acts upon the thyroid gland to stimulate the production of T4 and T3. The levels of t4 and T3 normally regulate the secretion of TSH by their negative feedback effect at the pituitary and possibly at the hypothalamus. Therefore, the measurement of circulation TSH levels is an important part of the investigation of disorders of the hypothalamic/pituitary/thyroid axis.

ADI's TSH ELISA kit is a very sensitive assay for the measurement of TSH in human serum.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on twenty replicate determinations of the zero standard, the minimum concentration of TSH detected using this assay is < 0.5 uIU/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Three serum samples (mean TSH concentrations 3.8, 13.3, and 45.5 uIU/ml) were run in ten replicates. The samples showed good intra-assay precision with %CV of 6.45, 3.63, and 4.43, respectively.

Inter-assay precision:

Three serum samples (mean TSH 3.59, 12.47, and 42.50 uIU/ml) were run in duplicate in ten independent assays. The samples showed good inter-assay precision (2-7 %CV). The actual values were: mean 3.59 uIU/ml, SD 0.28 uIU/ml, %CV 7.71; mean 12.47 uIU/ml, SD 0.32 uIU/ml, %CV 2.54; mean 42.5 uIU/ml, SD 1.08 uIU/ml, %CV 2.5, respectively.

3. RECOVERY

A known amount of TSH (5-30 uIU/ml) was added to a pooled patient sera and the total TSH concentrations measured. The assay showed excellent mean recoveries of about 96-105%.

4. LINEARITY

Pooled serum sample with original TSH concentrations of 44.0 uIU/ml was serially diluted (1:2 to 1:128) with the zero standard and their final TSH values determined. The samples showed excellent mean recoveries of about 98% (range 95-108%).

5. SPECIFICITY

The specificity of TSH ELISA kit was determined by measuring interference from high concentrations of hLH (up to 200 mIU/ml), hFSH (up to 200 mIU/ml), and HCG (up to 400 mIU/ml). These hormones had a minimal interference (TSH equivalent of <0.25 uIU/ml) in this assay.

6. HIGH DOSE HOOK EFFECT

TSH concentration of 250 uIU/ml did not cause any hook effect.

7. SPECIES REACTIVTY

Human TSH ELISA kit has not been tested for reactivity with TSH from other species (mouse, rat, etc).

References: Burger (1977) J Endocrinol. Metabol. 6, 83; Hamilton CR (1970) New England J. Med. 283, 1077; Lyon ICT (1984) New Eng. J. Med. 97, 175; Parker DC (1976) J. Clin. Endocrinol. Metab. 43, 318; Patel YC (1973) J. Clin. Endocrinol. Metab. 37, 190

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}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^{o}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 BEFORE USE).*

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. Label or mark the microtiter well strips to be used on the plate. Use **Sample diluent** for sample dilution only. Dilute **wash buffer 1:20** with distilled water (25 ml stock in 475 ml).

2. Pipet **50 µl of standards**, control, and serum samples into appropriate wells in *duplicate*.

3. Add **100 µl of enzyme conjugate** into each well**.** Mix gently.

4. Cover the plate and incubate for **60 minutes** at room temperature.

5. Aspirate and **wash the wells 3 times** with 300 µl wash buffer. We recommend using an automated ELISA plate Washer for better consistency and to avoid contamination. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

6. Dispense **100 ul TMB substrate per well**. Mix gently.

7. Cover the plate and incubate for **15 minutes** at room temperature.

8. Stop the reaction by adding **50 µl of stop solution** to all wells at the same timed intervals as in step 6. Mix gently.

9. Measure the **abs. at 450 nm** using an ELISA reader within 30 min.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{o}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Serum samples do not usually require dilution. However, if dilution is desired, the sample diluent must be used and the results obtained should be multiplied by the appropriate dilution factor. It may also be possible to use normal saline for sample dilution if sample diluent is not available.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on log-log graph paper by plotting net absorbance values of standards against appropriate LH concentrations. Read off the LH concentrations of the control and patient samples.

Expected Values

1. It is recommended that each laboratory determine its own normal and abnormal range.

2. As a guide from the literature, the value of adult TSH is < 6 uIU/ml. Other values are as follows.

Samples	N	Range	uIU/ml
Euthyroid	110	Upper Limit	5.5
Hyperthyroid	45	Less Than	0.6
Hypothyroid	20	Greater than	5.0

3. Some studies have indicated a circadian and episodic variation associated with TSH secretion. Newborn infants have substantially higher levels than are found later in life.

Indication for quantitative assay of TSH

1. Normal serum TSH level is generally < 6.0 uIU/ml. With most assay being unable to detect levels < 1.0 uIU/ml.

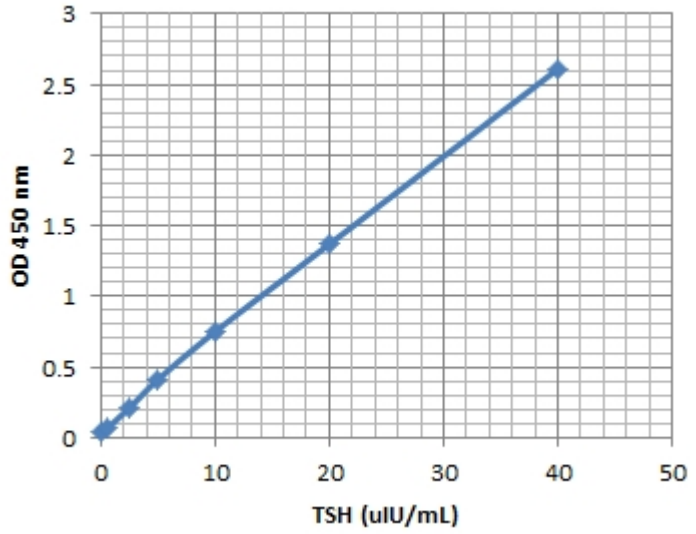
2. Primary hypothyroidism in thyroprivic and goitorous varieties: An elevated TSH levels is the most sensitive screen test for detecting hypothyroidism. The TSH level may be high before any clinical or laboratory test can show a low circulating level of T4.

3. Hypothalamic-pituitary hypothyroidism: The TSH level is undetectable in hypothalamic-pituitary hypothyroidism minimal elevation of thyroid hormones in the blood prevent the TSH release following administration of TRH. IN addition to possible evidence of intracranial disease hyposecretion of TSH is accompanied by hyposecretion of other pituitary hormones. A subnormal response of the serum TSH to the administration of TRH confirms the presence of hypothalamic-pituitary hypothyroidism.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A_{450nm}	Calculated Conc. (uIU/ml)
A1, A2	**Std. A** (0 uIU/ml)	0.039	
B1, B2	**Std. B** (0.5 uIU /ml)	0.07	
C1, C2	**Std. C** (2.5 uIU /ml)	0.29	
D1, D2	**Std. D** (5 uIU /ml)	0.49	
E1, E2	**Std. E** (10 uIU /ml)	1.00	
F1, F2	**Std. F** (20 uIU /ml)	1.66	
G1, G2	**Std. G** (40 uIU /ml)	2.60	

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.



Kit-spec-XL

A typical std. assay curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

Thyroid-stimulating hormone (TSH) ELISA kit is based on sequential binding of human TSH from samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of TSH present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. The unknown sample values are then read-off the standard curve.

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 TSH 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).

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

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 -20ºC for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

REAGENTS PREPARATION FOR THE ASSAY

Before use dilute **wash buffer 1:20** with distilled water (25 ml stock in 475 ml). Diluted buffer can be stored at 4oC for several weeks