

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

Instruction Manual No. M-1100

#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)		

TOTAL THYROXINE (TOTAL T₄)

ELISA KIT Cat. No. 1100, 96 Tests

**For Quantitative Determination of Total T₄
In Human Serum**

For In Vitro Research Use Only



4638 N Loop 1604 West • San Antonio • Texas 78249 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777

Email: Techsupport@4adi.com

ELISA KIT Cat. No. 1100 (96 tests)For Quantitative Determination of Total T₄ in Human Serum

Kit Components (96 tests)	Cat #
Mouse Anti-T ₄ Coated Strip plate, (96 wells)	1101
T ₄ Standard A , 10.0 ml; 0 µg%	1102
T ₄ Standard B , 0.5 ml; 1 µg%	1103
T ₄ Standard C , 0.5 ml; 4 µg%	1104
T ₄ Standard D , 0.5 ml; 12 µg%	1105
T ₄ Standard E , 0.5 ml; 32 µg%	1106
T ₄ Controls, Low & High , 0.5 ml, (LC1100-HC1100)	
Exact values of stds and control (lot specific) are provided on the vials. Stability: Approx. 12 months in unopened vial or as indicated on label. Once opened, the control should be used within 14 days or aliquot and stored frozen. Avoid multiple freezing and thawing cycles.	
Assay buffer , 25 ml	1108
T₄-HRP Conjugate (25X) , 1 ml	1109
Wash buffer 50 ml (10X)	W-10
TMB Substrate Soln , 16 ml	TMB-10
Stop Solution , 6 ml	T-10
Complete Instruction Manual	M-1100

Intended Use:

The Thyroxine (T₄) ELISA Kit is intended for the detection of Total T₄ in human serum. For **In Vitro research use only (RUO)**.

Introduction

The thyroid gland produces T₄, triiodothyronine T₃ and calcitonin. The first two hormones are synthesized by the gland following entrapment of iodine, conversion to iodine, and coupling of iodine with tyrosine, followed by coupling of two iodinated tyrosine molecules. T₄ and T₃ so formed are attached to thyroglobulin for storage and are released, as needed, as protease splits them from the globulin. Thyroxine is a highly active thyrometabolic hormone, which exists, in protein-bound and unbound forms. For T₄ can be measured more easily and with greater accuracy than T₃, determination of total T₄ by immunoassay is the most reliable and convenient screening test available for detecting thyroid disorders in man.

Release of T₄ and T₃ from the thyroid is greatly influenced by pituitary-thyroid stimulating hormone (TSH), which in turn is influenced by hypothalamic thyrotropin-releasing hormone (TRH). Normally, increased blood levels of T₄ and T₃ act to decrease the amount of TSH secreted, thereby reducing the production and release of T₄ and T₃. Decreased blood levels of T₄ and T₃ produce the opposite effect, leading to increased production and secretion of T₄ and T₃. In this manner a normal circulating thyroid hormone balance is maintained, circulating T₄ and T₃ are bound largely to thyroxine binding globulin (TBG). To a lesser extent they are bound to thyroxine binding prealbumin (TBPA) and, when present in excess, to albumin. Usually T₄ and T₃ concentration ratio is about 9:1, however T₃ has considerably greater physiological activity. It is the small free fraction (0.1% of the total or less) that is physiologically active and determines the clinical thyroid status of the patient's hyperthyroid, euthyroid, or hypothyroid.

PERFORMANCE CHARACTERISTICS**Sensitivity**

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the T₄ ELISA kit is 0.6 µg%.

Intra-Assay Precision <9.2%

Inter-Assay Precision <12.3%

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values

Group	Range (µg%)
Euthyroid	4–12
Hyperthyroid	> 12
Hypothyroid	< 4

Specificity (cross reactivity)

The following compounds were tested for cross-reactivity with the T₄ ELISA kit with T₄ cross-reacting at 100%.

Compound	% Cross Reactivity
L-Thyroxine	100
D-Thyroxine	94
3,3',5'-Triiodo-L-Thyronine (Reverse T ₃)	86
3,3',5'-Triiodo-L-Thyronine (T ₃)	3.3
3,3',5'-Triiodo-D-Thyronine	1.8
3,3',5'-Triiodothyropropionic acid	0.6

The following compounds were tested but cross-reacted at less than 0.04%: Acetylsalicylic acid, 3,5-Diiodo-L-Thyronine, 3,5-Diiodo-L-Tyrosine and 3-Iodo-L-Tyrosine.

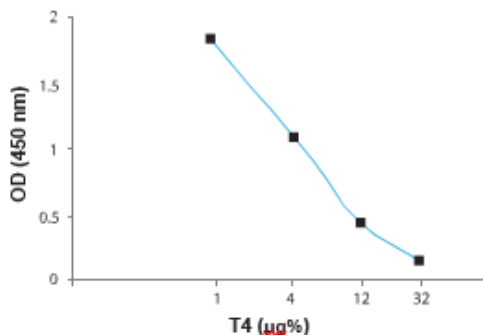
WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A _{450 nm}	Calculated Conc _n (ug %)
A1, A2	Std. A (0 µg%)	2.175	
B1, B2	Std. B (1 µg%)	1.835	
C1, C2	Std. C (4 µg%)	1.101	
D1, D2	Std. D (12 µg%)	0.451	
E1, E2	Std. E (32 µg%)	0.155	
F1, F2	Sample 1	0.635	8.3

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.

CALCULATION OF RESULTS

- Calculate the mean optical density of each calibrator duplicate.
- Draw a calibrator curve on semi-log paper with the mean optical densities on the Y- axis and the calibrator concentrations on the X- axis. If immunoassay software is being used, a 4-parameter curve is recommended.
- Calculate the mean optical density of each unknown duplicate.
- Read the values of the unknowns directly off the calibrator curve.
- If a sample reads more than 32 µg%, then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.



PRINCIPLE OF THE TEST

T₄ ELISA kit is based on competitive binding of human thyroxine from serum samples and enzyme-labeled T₄ to T₄-specific antibodies immobilized on microtiter well plates. After a washing step, chromogenic substrate is added, and color developed. The enzymatic reaction (blue color) is inversely proportional to the amount of T₄ present in the sample. The reaction is terminated by adding stopping solution (converts blue to yellow). Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of T₄ in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipette (20-100 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International T₄ ELISA test is intended for *in vitro* research use only.

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)

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera cannot 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.

REAGENTS PREPARATION:

Dilute **Wash buffer (10X)** in distilled water (Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water).

Prepare **1X solution of T₄-HRP conjugate**. Dilute 1:25 in assay buffer before use (eg. 80 µL of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 800 µL of HRP in 20 mL of assay buffer. Do not store diluted conjugate and prepare only in required amounts.

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 12 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8°C or stored frozen in small aliquots.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

1. Label or mark the microtiter well strips to be used on the plate. **Dilute the enzyme conjugate (1:25) with assay buffer and wash buffer (1:10) with water.** Dispense 200-300 ul of wash buffer or water to all wells. Mix for 5 seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.
2. Pipet **20 ul of standards**, control, and serum samples into appropriate wells in *duplicate*.
3. Add **150 ul of diluted enzyme conjugate** into each well. Mix gently for 10 seconds. Cover the plate and Incubate on a plate shaker (approximately 200 rpm) for **30 minutes at room temperature**.
4. Aspirate and **wash the wells 3 times** with 300 ul of 1x wash buffer. We recommend using an automated ELISA plate washer for better consistency. 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.
5. Add **150 ul TMB substrate**. Mix gently for 10 seconds. Cover the plate and Incubate on a plate shaker **for 15–20 minutes at room temperature** (incubation time may be decreased or increased by ~5 min to achieve optimal A450 of zero standard ~2.0-2.5).
6. Stop the reaction by adding **50 ul of stop solution** to all wells. Mix gently for 10 seconds.
7. Measure the **absorbance at 450 nm** using an ELISA reader within 20 min. yellow color will fade with time.

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°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.

Limitations:

1. All the reagents within the kit are calibrated for the direct determination of T4 in human serum. The kit is not calibrated for the determination of T4 in other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only **calibrator A may be used to dilute any high serum samples**. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/ products if false results are suspected.