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)

TOTAL THYROXINE (TOTAL T_4)

ELISA KIT Cat. No. 1100, 96 Tests

For Quantitative Determination of Total T_4 In Human Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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
Web Site: www.4adi.com

ELISA KIT Cat. No. 1100 (96 tests)
For Quantitative Determination of Total T_4 in Human Serum

Kit Components (96 tests)	Cat #
streptavidin coated Strip plate, (96 wells)	1101
T4 **Standard A**, 0.5 ml; 0 ug/dL	1102A
T4 **Standard B,** 0.5 ml; 2.0 ug/dL	1102B
T4 **Standard C**, 0.5 ml; 5.0 ug/dL	1102C
T4 **Standard D**, 0.5 ml; 10.0 ug/dL	1102D
T4 **Standard E**, 0.5 ml; 15.0 ug/dL	1102E
T4 **Standard F**, 0.5 ml; 25.0 ug/dL	1102F
Assay Diluent, 7 ml	1105AD
T4-**HRP Conjugate** conc. (11X), 0.7 ml,	1103
Anti-**T4 Biotin Solution** ; 7 ml	1104
HRP **Substrate** Solution, 12 ml	1100-SS
Wash Buffer (20X); 25 ml, dilute with 475 ml of distilled water	1 1 0 0 - W B
Stop Solution, 12 ml	1100-ST
Complete Instruction Manual	M-1100

Intended Use:

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

Introduction

The thyroid gland produces T4, trilodothyronine 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.

Inter-assay precision:

Three serum samples (4-15 ug/dL) were run in duplicate in sixteen independent assays. The samples showed good inter-assay precision (2.7-4 % CV).

3. RECOVERY

A known amount of total T_4 (3-24 ug/dL) was added to three patient sera (with original total T_4 concentrations of 3 and 6 ug/dL) and the final total T_4 concentration measured. The assay showed good mean recoveries of about 93% (range 85-96%).

4. LINEARITY

A serum sample containing 24 ug/dL was diluted with a series of T4-free serum. The dilutions were tested and the T4 recoveries were compared with the expected concentration. The samples showed excellent mean recoveries of about 102% (range 90-109%).

5. SPECIFICITY

The specificity of total T_4 ELISA kit was determined by measuring interference from high concentrations of 3,5-diiodothyronine (up to 10 ug/dL), 3,3',5-triiodothyronine (rT_3,up to 1000 ng/ml), 3,3',5-triiodothyronine (T_3,up to 59 nmol/l), 3,3',5-triiodothyroacetic acid (up to 42 nmol/l), 3,3',5-triiodothyropropionic acid (up to 63 nmol/l), Aspirin (10 mg/dL) iodoacetic acid (10 ug/dL), phenylbutazone (10 mg/dL). No significant cross-reaction was observed with any of these compounds.

6. CORRELATIVE STUDY

ADI total T4 elisa kit was compared with Inc Star Clinical assay T4 RIA by analyzing 99 samples (2.6-25.5 ug/dL). The regression analyses showed good correlation between the two assays.

	Inc Star RIA	ADI T4 ELISA
Mean Sample (ug/dL)	8.682	8.854

Slope = 0.98288; Intercept =0.790 Correlation Coefficient =0.852
ADI = 0.929 (Inc stat T4)+ 0.790

7. SPECIES REACTIVITY AND PUBLICATIONS

T4 is the same in human and animals. ADI human T4 elisa Kit has been used in mouse and rat samples (see below).

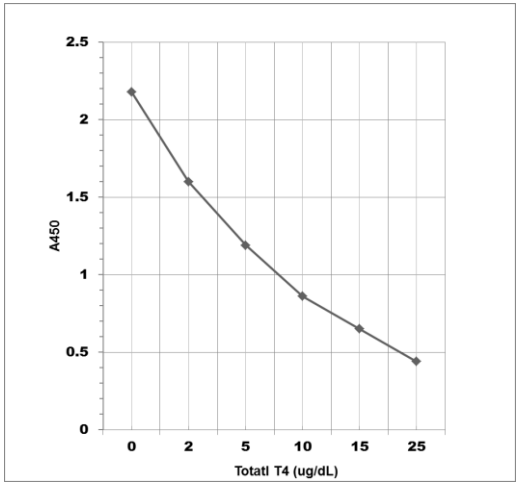
Maglich 2004, J. Biol. Chem., 279, 19832 – 19838, mice serum T4
Huang W, 2005, J. Nutr., 135: 1631 - 1635 T4 ELISA
Xiao CW, 2004, J. Nutr., 134: 743 – 749, T4 in rat Plasma

General Refrences: Ekins R et al (1969) Clin. Biochem. 2; Skelly D et al (1973) Clinc. Chem. 2, 19; Shcuurs A et al (1977) Clin. Chim. Acta 5, 81; Scharpe S et al (1976) Clin. Chem. 6, 22; Wisdom G et al (1976) Clin Chem. 8, 22, Schall R et al (1978) Clin. Chem. 10, 24;

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean $A_{450\ nm}$	A/A0 x 100 Index	Calculated Concn (ug d/L)
A1, A2	**Std. A** (0 ug/dL)	2.679		
B1, B2	**Std. B** (2 ug/dL)	1.961		
C1, C2	**Std. C** (5 ug/dL)	1.322		
D1, D2	**Std. D** (10 ug/dL)	0.901		
E1, E2	**Std. E** (15 ug/dL)	0.659		
F1, F2	**Std. F** (25 ug/dL)	0.396		
G1, G2	**Sample 1**			

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.



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

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on sixteen replicates determinations of the zero standard, the minimum concentration of total T_4 detected using this assay is 0.5 ug/dL. 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 total T_4 concentrations 3.5, 6.8, 13.9 ug/dL) were run in five separate runs. The samples showed good intra-assay precision with %CV of 9-11.

PRINCIPLE OF THE TEST

Total T_4 ELISA kit is based on competitive binding of human thyroxine from serum samples and enzyme-labeled T_4 to T_4-specific antibodies immobilized on microtiter well plates. In the assay, total T_4 is released from its binding proteins by a releasing agent present in the assay buffer. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (blue color) is inversely proportional to the amount of T_4 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 T_4 in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

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

PRECAUTIONS

The Alpha Diagnostic International Total T_4 ELISA test is intended for *in vitro research* use only. The reagents contain proclin-300 as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses; therefore, sera should be handled with appropriate precautions.

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 cannot be immediately assayed, these could be stored at -20^{O}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 (1:20) with distilled water (25 ml stock in 475 ml). Store at 4oC

Enzyme conjugate (11X) dilute 1:11 with assay diluent. (For example, dilute 160µl of enzyme conjugate with 1.6ml of assay diluent for 16 wells (A slight excess of solution is made). This reagent should be used within twenty-four hours for maximum performance of the assay. Store at 2-8°C. **Dilute conjugate in required amounts only and do not store diluted conjugate.**

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 under appropriate storage conditions. The unused portions of the standards should be stored at 2-8^{o}C or stored frozen in small aliquots.

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. Dilute conjugate in required amounts. **Dilute wash buffer (1:20) with distilled water (25 ml stock in 475 ml).** Store at 4oC.

1. Label or mark the microtiter well strips to be used on the plate. Store unused strips in the sealed pouch at 4oC.

2. Pipet **25 ul of standards**, control, and serum samples into appropriate wells in *duplicate*.

3. Add **50 ul of enzyme conjugate** into each well**.** Add **50 ul of T4-Antibody biotin Solution** to all wells. Mix gently for 20-30 seconds. Cover the plate and incubate for **60 minutes** at room temperature.

4. Aspirate and **wash the wells 3 times** with **300 ul** of 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. Dispense **100 ul TMB substrate per well.** Mix gently for 5-10 seconds.

6. Cover the plate and incubate for **15 minutes** at room temperature. Blue color develops in standards and samples.

7. Stop the reaction by adding **50 ul of stop solution** to all wells at the same timed intervals as in step 6. Mix gently for 5-10 seconds. Blue color turns yellow.

8. Measure the **absorbance 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.

Limitations

Serum samples containing more than 24 ug/dL T_4 should be diluted with the zero standard (standard A or normal saline) and the results obtained should be multiplied by the appropriate dilution factor. Samples containing T4 <0.5 ug/Dl are analyzed by diluting with 2 ug/dL T4 calibrator to extend the curve.

Calibrators and controls from other manufacture should not be used as they may contain serum preservatives incompatible with ADI's ELISA reagents.

Whenever laboratory data conflicts with clinical findings or impressions, clinical judgment should be exercised and additional evaluations undertaken.

Use of ADI's reagent in a study of euthyroid patients in one geographic location will yield a normal range. It is recommended that laboratories adjust normal values to reflect geographic and population differences specific to the patients they serve.

CALCULATION OF RESULTS

Draw the standard curve on a linear graph paper by plotting net absorbance values of standards against appropriate total T_4 concentrations. Read off the total T_4 concentrations of the control and patient samples.

EXPECTED VALUES

In a study of 75 euthyroid patients yielded a normal range of (4-12 ug/dL) at the 95% confidence limit. It is recommended that researchers adjust normal values to reflect geographic and population differences.