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		

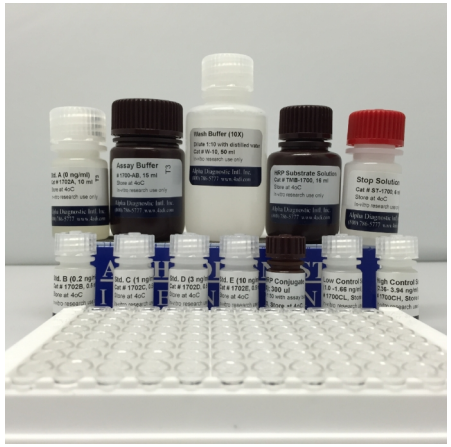
#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-1700

Total Triiodothyronine (Total T3)

ELISA KIT Cat. No. 1700

For Quantitative Determination of Total T3 In Human Serum



For In Vitro Research Use Only

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

ELISA KIT Cat. No. 1700 (96 tests)

For Quantitative Determination of Total T3 in Human Serum

Kit Components (96 tests)	Cat #
Anti-T3 Coated Strip plate, (96 wells)	1701
T3 **Standard A**, 10 ml; 0 ng/ml	1702A
T3 **Standard B**, 0.5 ml; 0.2 ng/ml	1702B
T3 **Standard C**, 0.5 ml; 1 ng/ml	1702C
T3 **Standard D**, 0.5 ml; 3 ng/ml	1702D
T3 **Standard E**, 0.5 ml; 10 ng/ml	1702E
T3 controls, **Low & High** (exact values printed on vials); 0.5 ml	
T3-HRP Conjugate (50X). 0.3 ml, dilute 1:50 with assay buffer	1703
Assay Buffer, 15 ml	1700-AB
HRP Substrate Solution, 16 ml	TMB-1700
Wash Buffer (10X); 50 ml, **dilute 1:10 with distilled water**	W - 1 0
Stop Solution, 6 ml	ST-1700
Complete Instruction Manual	M-1700

Introduction

The thyroid gland produces thyroxine 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.

Measurement of serum levels of triiodothyronine (T3) is an important adjunct in the determination of thyroid function. An elevated serum is a strong indication of hyperthyroidism. In patients who demonstrate clinical hyper-thyroidism, an elevated serum triiodothyronine level combined with a normal serum thyroxine level is evidence for T3 thyrotoxicosis, most patients with unequivocal clinical hypothyroidism, levels of triiodothyroine are below normal. However, in borderline cases, Triiodothyronine levels are sometimes or often normal. Thus, a reduced serum level of triiodothyronine is not so clear an indicator or primary hypothyroidism as a reduced serum level of thyroxine or an elevated level of thyroid-stimulating hormone.

3. RECOVERY

A known amount of total T3 was added to three patient sera (with original total T3 concentrations of 1.3 ng/ml) and the final total T3 concentration measured. The assay showed good mean recoveries of about 95% (range 90-115%).

4. LINEARITY

Three serum samples (2.9, 5.1, 8.0 ng/ml) were diluted (1:2, 1:4, 1:8) with a T3-free serum. The dilutions were tested and the T3 recoveries were compared with the expected concentration. The samples showed excellent mean recoveries of about 105% (range 97-115%).

5. SPECIFICITY

The specificity of total T3 ELISA kit was determined by measuring interference from high concentrations of T4, 3,3', 5-triiodothyronine (rT_3, up to 1000 ng/ml), 3,5-diiodothyronine (up to 10 ug/dL), Aspirin (10 mg/dL) iodoacetic acid (10 ug/dL), phenylbutazone (10 mg/dL), 3',5-diiodothyronine (T2), and sodium salicylate. No significant cross-reaction was observed with any of these compounds.

Species Crossreactivity

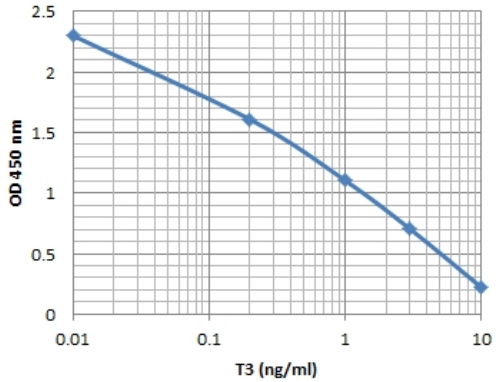
T3 and T4 are the same in all species. The ELISA kit #1700 has been used in mouse and rat and also in cell culture medium and plasma. Please consult the following publications using ADI ELISA kit #1700.

Belakavadi M	2011	Mol. Cell. Endocrinol. 339, 72-80
Andersson L	2011	Endocrinology, 152: 1154 - 1164.
Lin Y	2011	J. Endocrinol., May 2011; 209: 185 - 191.
You S-H	2010	Mol. Endocrinol., Jul 2010; 24: 1359 - 1367
Machado D S	2009	PNAS, Jun 2009; 106: 9441 - 9446.
Chen Y-F	2010	Am J Physiol Heart Circ Physiol, 298: H259 - H262.
Depke M	2008	Endocrinology, Mar 2008; doi:10.1210/en.2008-0038
Ohguchi H	2008	Mol. Cell. Biol., Apr 2008; doi:10.1128/MCB.02154-07
Mesaros A	2008	Cell Metabolism, 7, 236-248
Wojcikowski J	2008	Biochemical Pharmacology, 76, 258-267
Halapas A	2007	Experimental Physiology, 93, 237-246
Guijarro A	2007	Am J Physiol Regul. Integ. Comp Physiol, 293: R1474 - R1489
Pantos C	2007	Eur. J. Endocrinol., Apr 2007; 156: 415 - 424.
Pantos C	2007	Eur. J. Cardiothorac. Surg., Aug 2007; 32: 333 - 339
Huang W	2005	J. Nutr., 135: 1631 - 1635
Xiao CW	2004	J. Nutr., 134: 743 - 749
Tsai Chen-En	2002	Current Biol. 12: 1221-1226

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean $A_{450\ nm}$
A1, A2	**Std. A** (0 ng/ml)	2.467
B1, B2	**Std. B** (0.2 ng/ml)	1.998
C1, C2	**Std. C** (1 ng/ml)	1.134
D1, D2	**Std. D** (3 ng/ml)	0.554
E1, E2	**Std. E** (10 ng/ml)	0.223
F1, F2	**Sample 1**	1.074

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values.



Kit-specs-XL

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 T3 detected using this assay is **0.16 ng/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 total T3 concentrations 0.645 1.19, 5.16 ng/mL) were run in three separate runs. The samples showed good intra-assay precision with %CV of 4.1-11.

Inter-assay precision:

Three serum samples (0.64, 1.24, 4.86, 290, and 665 ng/dL) were run in duplicate in sixteen independent assays. The samples showed good inter-assay precision (3.0-10% CV).

PRINCIPLE OF THE TEST

Total T3 ELISA kit is based on competitive binding of human thyroxine from serum samples and enzyme-labeled T3 to T3-specific antibodies immobilized on microtiter well plates. In the assay, total T3 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 T3 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 T3 in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

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

PRECAUTIONS

The Alpha Diagnostic International Total T3 ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal or Kathon 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 Proclin-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.

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. Do not touch the bottom of the wells.

REAGENTS PREPARATION

Dilute wash buffer (1:10) with distilled water (50 ml stock in total of 450 ml water). Store at 4oC.

Dilute HRP conjugate conc. 1:50 in assay buffer (40 ul of HRP in 2 ml of assay buffer)

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 wash buffer (1:10) with distilled water (50 ml stock in 450 ml). Dilute HRP conjugate conc. 1:50 in assay buffer (40 ul of HRP in 2 ml of assay buffer)**

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

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

3. Cover the plate and incubate on a plate shaker (200 rpm approx.) for **60 minutes** at room temperature.

4. Aspirate and **wash the wells 3 times** with 300 µl 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 **150 ul TMB substrate per well**. Mix gently.

6. Cover the plate and incubate for **15 minutes** at room temperature. Blue color develops (incubation time can be varied + so as to get std A A450=2.00-2.500).

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

8. Measure the **absorbance at 450 nm** using an ELISA reader within 30 min. **Note**: If A450 exceeds the upper limit of detection, it is possible to read the plate ad 405 nm or 415 nm. Optical density will be lower but this will not impact the results.

Limitations

Serum samples containing >1000 ng/dL T3 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 T3 <50 ng/dL are analyzed by diluting with 0 ng/dL T3 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

Calculate the index A450 values of standards and samples versus its concentration. Draw the standard curve on a semi-log graph paper by plotting net absorbance values of standards against appropriate total T3 concentrations. Read off the total T3 concentrations of the control and patient samples. If using an immunoassay software, a 4-parameter curve is recommended.

EXPECTED VALUES

1. In a study of healthy normal males and females T3 values were 0.7-2.1 ng/ml.

2. In an individual having normal levels of TBG, the measurement of total T3 yields an accurate diagnosis of thyroid status. However, there are many circumstances in which the levels of TBG are not normal. For instance, pregnancy or estrogen therapy cause increases in total T3, whereas androgenic steroids have the opposite effect. Because of this variation in TBG levels, interpretation of T3 results should be tempered by the determination of TBG binding capacity.

3. Depressed levels of T3 have been observed in a wide variety of serous, nonthyroidal illness such as hepatic cirrhosis, anorexia nervosa, chronic renal failure, and disseminated malignancy after surgery and during caloric restriction.

4. Zero standard absorbance will decrease gradually with age of the conjugate. A sudden large change in A450 and/or a large shift in the standard curve may indicate problems with the assay or components.