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-1650

Free Triiodothyronine (fT3)

ELISA KIT Cat. # 1650

For Quantitative Determination of Free T3
In Human, Monkey, Mouse, Rat or other animals Serum

For In Vitro Research Use Only (RUO)

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

ELISA KIT Cat. No. 1650 (96 tests)
For Quantitative Determination of Free T3 in Serum

Kit Components(96 tests)	Cat #
Anti-fT3 Coated Strip plate, (96 wells)	1651
fT3 **Standard A**, 0.5 ml; 0 pg/ml	1652A
fT3 **Standard B**, 0.5 ml; 2 pg/ml	1652B
fT3 **Standard C**, 0.5 ml; 4 pg/ml	1652C
fT3 **Standard D**, 0.5 ml; 8 pg/ml	1652D
fT3 **Standard E**, 0.5 ml; 16 pg/ml	1652E
fT3 **Standard F**, 0.5 ml; 40 pg/ml	1652F
fT3 **Controls Low & High** 0.5 ml (lot sp. Values are given on the vial); #1653CL-CH	
Exact values of stds and control (lot specific) are provided on the vials. Stability: 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, 15 ml	1654
fT3-HRP **Conjugate**, 300 ul (50X)	1655
Wash buffer 50 ml (10X)	W-10
TMB Substrate Soln, 16 ml	TMB-10
Stop Solution (1M sulfuric acid), 6 ml	T-10
Complete Instruction Manual	M-1650

Intended Use

Free Triiodothyronine (T3) is a competitive ELISA for the determination of free T3 in human, monkey, or animal sera. For research use only (RUO), not for therapeutic use.

Introduction

Triiodothyronine (T3) is a thyroid hormone found circulating in the bloodstream. T3 contains three iodine atoms and is produced largely through the extrathyroidal conversion of thyroxine (T4), the principal thyroid hormone with four iodine atoms. Most of the T3 that circulates in the blood is bound to carrier proteins such as TBG, pre-albumin and albumin. The free fraction of T3 (fT3), which represents only 0.25% of the total amount, is considered to be the physiological active fraction.

Total T3 levels depend not only on thyroid status and the peripheral conversion of T4 to T3, but also on the concentration of thyroid hormone-binding proteins. Free T3 (fT3) on the other hand, is largely unaffected by variations in these carrier proteins which can occur under conditions such as pregnancy, estrogen therapy and the use of oral contraceptives. Therefore, free T3 typically reflects a patient's actual thyroid status more reliably than total T3.

Measurement of free T3 is generally recommended for patients with symptoms of hyperthyroidism as found in Graves' disease, toxic adenoma and toxic multinodular goiter.

INTRA-ASSAY PRECISION

Three samples (5.18, 8.5, 48.2 pg/ml) were assayed ten times each on the same calibrator curve. The values were SD 0.50, 0.59, 1.68 pg/ml and CV% 9.7, 7.0, 3.5 respectively.

INTER-ASSAY PRECISION

Three samples (3.30, 5.15, 9.69, pg/ml) were assayed ten times each on the same calibrator curve. The values were SD 0.28, 0.40, 0.71 pg/ml and CV% 8.6, 7.8, and 8.2% respectively.

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. The following reference range (pg/ml) was established with 80 apparently healthy adults: Normal Euthyroid Samples (N=44; mean=3.7; range 2.2-5.3 pg/ml).

EFFECT OF THYROXINE BINDING GLOBULIN (TBG)

The purpose of this study was to investigate a possible interference caused by the binding of TBG to the fT3-HRP conjugate. The zero calibrator was spiked with purified TBG and assayed. The results are tabulated below:

TBG (µg/ml added)	OD	% B/B_0
0	1.255	100
12.5	1.229	98
25	1.170	93
50	1.137	91
100	1.168	93
200	1.174	94
400	1.118	89

The results show no binding of labeled T3 to TBG even at higher than normal levels. In conclusion, results showed that there was no significant influence by TBG in the Direct Free T3 Direct ELISA Kit.

EFFECT OF HUMAN SERUM ALBUMIN (HSA)

The purpose of this study was to investigate a possible interference of HSA on the assay procedure. The zero calibrator was spiked with purified HSA and assayed. The results are tabulated below:

HSA (mg/ml added)	OD	% B/B_0
0	1.255	100
3.125	1.228	98
6.25	1.331	100
12.5	1.245	99
25	1.197	95
50	1.217	97
100	1.063	85

The results show no significant binding of labeled T3 to HSA even at higher than normal levels.

EFFECT OF NON-ESTERIFIED FATTY ACIDS (NEFA)

The purpose of this study was to investigate a possible interference of NEFA on the assay procedure. Two samples were spiked with oleic acid and assayed. The results are tabulated below:

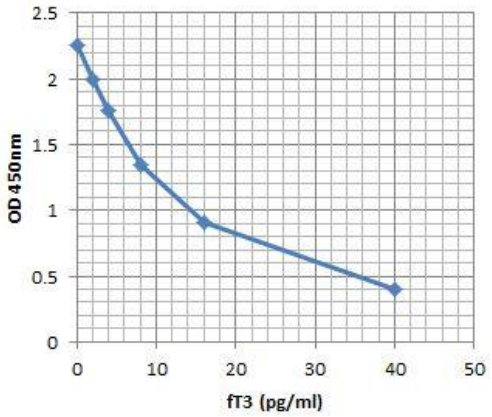
NEFA (mmol/L added)	Sample 1 (pg/ml)	Sample 2 (pg/ml)
0	4.4	8.7
0.5	4.6	7.5
3.5	4.6	8.3
25	4.6	10.9

The results show that NEFA may increase the free T3 values, only at higher than normal concentrations.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A450 nm	Calculated Concn (pg/ml)
A1, A2	**Std. A** (0 pg/ml)	2.379	
B1, B2	**Std. B** (2 pg/ml)	2.152	
C1, C2	**Std. C** (4pg/ml)	1.863	
D1, D2	**Std. D** (8 pg/ml)	1.65	
D1, D2	**Std. E** (16 pg/ml)	1.366	
E1, E2	**Std. F** (40 pg/ml)	0.727	
F1, F2	**Sample 1**	1.760	3.8

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



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 direct fT3 ELISA kit is 0.3 pg/ml.

SPECIFICITY (CROSS REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct fT3 ELISA kit with T3 cross-reacting at 100%.

Compound	%Cross Reactivity
L-Triiodothyronine	100
D-Triiodothyronine	34
Triiodothyropropionic acid	20
Diiodo-D-thyronine	0.5
D-Thyroxine	0.3
L-Thyroxine	0.9

The following compounds were tested but cross-reacted at less than 0.1%: Diiodotyrosine, Iodotyrosine, Phenytoin, Sodium Salicylate and r-Triiodothyronine.

PRINCIPLE OF THE TEST

Free T3 ELISA kit is based on competitive binding of human free thyroxine from serum samples and enzyme-labeled T3 to T3-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 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 free T3 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 shaker (orbital shaker), plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International Free T3 ELISA test is intended for *in vitro research* use only. The reagents contain prolcin-300 (0.1% v/v) as preservative; necessary care should be taken when disposing solutions. The Control Serum may contain 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 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^{o}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

REAGENTS PREPARATION

Wash buffer Concentrate (10X): Prepare 1X solution by diluting 1:10 (50 ml concentrate in 450 ml water). Store diluted stock at 4oC.

Prepare **1X solution of fT3-HRP conjugate**. Dilute 20 ul stock conjugate per ml of assay buffer (200 ul in 10 ml for complete 96-well plate). 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. Once the procedure has been started, all the steps should be completed without interruption.

1. Label or mark the microtiter well strips to be used on the plate. **Dilute the enzyme conjugate (1:50) with assay buffer** and **wash buffer (1:10) with water**.

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

3. Add **100 ul of diluted enzyme conjugate** into each well**.** Mix gently for 10 seconds manually. Cover the plate and incubate for **60 minutes** at 37oC.

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 for **10-15 minutes** at 37oC.
   Note: if the absorbance of the zero standards is >2.5 then it is recommended to stop the color development at <15 min (e.g., 10 min) or increase the incubation time until zero standard reading is ~2.0.

6. 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 10 seconds.

7. Measure the **absorbance at 450 nm** using an ELISA reader within 30 min. Yellow color will slowly fade if plates are not read immediately.

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

1. All the reagents within the kit are calibrated for the direct determination of fT3 in human serum. The kit is not calibrated for the determination of fT3 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. Samples reading higher than 40 pg/ml should be reported as such and should not be diluted. Dilution will alter the existing equilibrium and may lead to false results.
5. The interpretation of free T3 results can be complicated by a variety of drugs, severe nonthyroidal illness and some rare conditions such as familial dysalbuminemic hyperthyroxinemia (FDH). For diagnostic purposes, the results of this assay should always be used in combination with the clinical examination, medical history and other findings.

CALCULATION OF RESULTS

1. Calculate the mean optical density of each calibrator duplicate.
2. 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.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.

Performance characteristics (continued from page 6)

EFFECT OF LIPEMIA

The purpose of this study was to investigate a possible interference of lipemic samples on the assay procedure. Two samples were spiked with triglycerides and assayed. The results are tabulated below:

Triglycerides (mg/dl added)	Sample1 (pg/ml)	Sample2 (pg/ml)
0	4.4	8.7
50	5.5	9.9
75	5.9	10.8

Results show that lipemic samples may increase the free T3 values. Therefore, lipemic samples should not be used in this assay.