

# Human T4 ELISA Kit

For the quantitative *in vitro* determination of Human Thyroxine (T4) concentrations in human serum samples

Catalogue Number: EL10007

*96 tests*

FOR LABORATORY RESEARCH USE ONLY.  
NOT FOR USE IN DIAGNOSTIC PROCEDURES.



2355 Derry Road East, Unit 23  
Mississauga, Ontario  
CANADA L5S 1V6

Tel: (905) 677-9221 or (877) 755-8324

Fax: (905) 677-0023

Email: [info@anogen.ca](mailto:info@anogen.ca) ♦ Web Site: [www.anogen.ca](http://www.anogen.ca)

## TABLE OF CONTENTS

	<b>Page</b>
INTENDED USE	2
INTRODUCTION	2
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	2
REAGENTS PROVIDED	3
MATERIALS REQUIRED BUT NOT SUPPLIED	4
PRECAUTIONS	4
SAMPLE COLLECTION AND STORAGE	4
PREPARATION OF REAGENTS	4
ASSAY PROCEDURE	5
CALCULATION OF RESULTS	6
TYPICAL DATA	7
.....Example	7
PERFORMANCE CHARACTERISTICS	7
.....Sensitivity	7
.....Intra-assay precision	7
.....Inter-assay precision	7
.....Accuracy	8
.....Recovery	8
.....Specificity	8
QUALITY CONTROL	8
REFERENCES	9

## **INTENDED USE**

Enzyme immunoassay (EIA) permits the routine quantitative determination of many protein hormones in body fluids and provides an accurate, sensitive, reproducible, rapid and specific assay. This enzyme immunoassay method makes it possible to measure very low concentration of T4 (Thyroxine) in small volumes of serum (50µL per assay).

## **INTRODUCTION**

Thyroxine (T4) and triiodothyronine (T3) are secreted from the thyroid gland and regulated by a sensitive feedback system involving the hypothalamus and pituitary gland. The hypothalamus releases the thyrotropin releasing hormone (TRH), which stimulates the pituitary to release the thyroid stimulating hormone (TSH). This causes the thyroid to release T3 and T4 and these in turn regulate the release of TRH and TSH via a feedback control mechanism. Thyroxine levels are generally found to be high in the serum of untreated patients with hyperthyroidism. Therefore, T4 may act as an indicator of the thyroidal state. Circulating T4 is almost exclusively bound by TBG. In order to quantitate total thyroxine in serum, the T4 must first be released from the native serum binding protein. This protein must also be inhibited from further participation in the assay.

## **PRINCIPLES OF THE ASSAY**

The ANOGEN Coated well immunoenzymatic assay for the quantitative measurement of serum T4 utilizes a solid phase coupled antibody and a conjugated T4. The sample to be assayed is incubated with the solid phase coupled antibody and conjugated T4. The conjugated T4 competes with T4 from the sample for available binding sites on the antibody.

After the incubation period, the wells are decanted. Both conjugated and unconjugated T4 bound to the antibody during the incubation remain on the solid phase. The substrate and the stopping solution are added to provide a color. The wells are counted in a microplate reader.

Standards of known T4 concentrations are run concurrently with the samples being assayed and a standard curve is plotted. The unknown T4 concentration in each sample is calculated from this curve.

## **LIMITATIONS OF THE PROCEDURE**

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a strict adherence to the exact procedure described within this package insert and good laboratory practice.
2. The T4 concentration should be used only as an adjunct to other data (ex. results of other tests, clinical impressions, etc.) available to the physician who can take into consideration the history of the patient. Each laboratory should compile its own normal ranges, if possible. This kit is suitable for use with serum of human origin only.
3. A maximal total pipetting time of ten (10) minutes per run is suggested.

## REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to expiration date on the label.

<b>96 tests</b>	
1. <b>MICROTITER PLATE</b> (Part EL07-1) _____	<b>96 wells</b>
Pre-coated wells with anti-T4 monoclonal antibody immobilized into the well.	
2. <b>ENZYME CONJUGATE (100X)</b> (Part EL07-2) _____	<b>0.5 mL</b>
Concentrated T4-HRP conjugate in stabilizer solution.	
3. <b>CONJUGATE DILUENT</b> (Part EL07-3) _____	<b>15 mL</b>
PBS buffer pH 7.2 with preservative.	
4. <b>T4 STANDARD – 18 ug/dL</b> (Part EL07-4) _____	<b>0.5 mL</b>
Prepared with human T4 in normal human serum containing preservative.	
5. <b>T4 STANDARD – 12 ug/dL</b> (Part EL07-5) _____	<b>0.5 mL</b>
Prepared with human T4 in normal human serum containing preservative.	
6. <b>T4 STANDARD – 9 ug/dL</b> (Part EL07-6) _____	<b>0.5 mL</b>
Prepared with human T4 in normal human serum containing preservative.	
7. <b>T4 STANDARD – 6 ug/dL</b> (Part EL07-7) _____	<b>0.5 mL</b>
Prepared with human T4 in normal human serum containing preservative.	
8. <b>T4 STANDARD – 3 ug/dL</b> (Part EL07-8) _____	<b>0.5 mL</b>
Prepared with human T4 in normal human serum containing preservative.	
9. <b>T4 STANDARD – 0 ug/dL</b> (Part EL07-9) _____	<b>0.5 mL</b>
Prepared with human T4 in normal human serum containing preservative.	
10. <b>SUBSTRATE A</b> (Part EL07-10) _____	<b>11 mL</b>
Buffered solution with H <sub>2</sub> O <sub>2</sub> .	
11. <b>SUBSTRATE B</b> (Part 30007) _____	<b>11 mL</b>
Buffered solution with TMB.	
12. <b>WASH BUFFER (20X)</b> (Part 30005) _____	<b>60 mL</b>
Concentrated solution of saline phosphate buffer with a preservative.	
13. <b>STOP SOLUTION</b> (Part EL30008) _____	<b>14 mL</b>
2 N Sulfuric Acid (H <sub>2</sub> SO <sub>4</sub> ). <b>CAUTION: Caustic Material!</b>	

## MATERIALS REQUIRED BUT NOT SUPPLIED

1. Precision pipettes (50  $\mu$ L) with disposable tips or a SMI pipette
2. 8 channels pipette (100 and 150  $\mu$ L) with disposable tips
3. Microplate reader with filter 450 nm
4. 8 channels repeater pipette
5. Deionized or distilled water
6. Absorbent paper

## PRECAUTIONS

1. All materials in this kit may be used only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material to human or animals.
2. The Standards and Enzyme Conjugate contain products derived from human blood. Handle the materials as though they were capable of transmitting infectious diseases, since no known test method can offer absolute assurance that such products will not transmit infectious agents even tested non-reactive.
3. Optimal results will be obtained by strict adherence to this protocol. Respect laboratory quality control rules.
4. The stopping solution contains sulfuric acid. This solution should be handle with caution, avoiding contact with skin.
5. Prior to assay, warm all reagents to ambient temperature by allowing them to stand at room temperature. Gently mix all reagents.

## SAMPLE PREPARATION

Serum must be used in this T4 procedure. No additive or preservative are necessary to maintain the integrity of the specimen. Store at 2-8°C and assay within one week after collection. If the assay cannot be performed within one week, freezing is recommended.

## PREPARATION OF REAGENTS

1. **Washing Solution (1X):** Dilute 1 volume of Wash Buffer (20X) with 19 volumes of deionized or distilled water. Wash buffer is stable for 1 month at 2-8°C. **Mix well before use.**
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes within 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	2.0	2.0	4.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **Enzyme Conjugate (1X):** Just before use, dilute the Enzyme Conjugate (100X) with Conjugate Diluent in the proportion of 1 volume of Enzyme Conjugate (100X) in 100 volumes of Conjugate Diluent (Dilution 1/100). Since the diluted Enzyme Conjugate (1X) is not stable, prepare just enough for the required number of tests. Suggested volume of diluted Enzyme Conjugate (1X) per number of wells:

No. of wells	Volume (mL) Enzyme Conjugate (100X)	Volume (mL) Conjugate Diluent
24	0.03	3.0
48	0.06	6.0
72	0.09	9.0
96	0.12	12.0

**Discard unused portion of diluted T4-HRP conjugate after completing the addition of the reagent to the wells.**

### ASSAY PROCEDURE

- DO NOT INTERCHANGE REAGENTS BETWEEN KITS BEARING DIFFERENT LOT NUMBERS.
  - ALL REAGENTS AND PATIENT SAMPLES SHOULD BE BROUGHT TO 22 ± 2°C BEFORE ASSAYING.
  - ALL REAGENTS AND PATIENT SAMPLES SHOULD BE MIXED BY SWIRLING OR GENTLY VORTEXING. DO NOT INDUCE FOAMING.
1. Prepare Wash Buffer and T4 Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards and Samples to the Microtiter Plate.*

Wells	Contents	Wells	Contents
<b>1A, 2A</b>	Standard 1 – <b>18 ug/dL</b> (S1)	<b>1E, 2E</b>	Standard 5 - <b>3 ug/dL</b> (S5)
<b>1B, 2B</b>	Standard 2 – <b>12 ug/dL</b> (S2)	<b>1F, 2F</b>	Standard 6 - <b>0 ug/dL</b> (S6)
<b>1C, 2C</b>	Standard 3 – <b>9 ug/dL</b> (S3)	<b>1G, 2G</b>	Blank (S7)
<b>1D, 2D</b>	Standard 4 – <b>6 ug/dL</b> (S4)	<b>1H-12H</b>	<b>T4 samples</b>

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	S1	S1	3	11	19	27	35	43	51	59	67	75
<b>B</b>	S2	S2	4	12	20	28	36	44	52	60	68	76
<b>C</b>	S3	S3	5	13	21	29	37	45	53	61	69	77
<b>D</b>	S4	S4	6	14	22	30	38	46	54	62	70	78
<b>E</b>	S5	S5	7	15	23	31	39	47	55	63	71	79
<b>F</b>	S6	S6	8	16	24	32	40	48	56	64	72	80
<b>G</b>	Blank	Blank	9	17	25	33	41	49	57	65	73	81
<b>H</b>	1	2	10	18	26	34	42	50	58	66	74	82

2. Add 50µL of T4 Standard or Sample to the appropriate wells.

3. Add 100 $\mu$ L of Enzyme Conjugate (1X) to the appropriate wells. Mix well. Cover and Incubate for 1 hours at 37°C.
4. Prepare Substrate Solution no more than 15 minutes before end of incubation (see Preparation of Reagents).
5. Wash the Microtiter Plate using one of the specified methods indicated below:  
Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Solution (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.  
Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Solution (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 $\mu$ L/well/wash (range: 350-400 $\mu$ L). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.
6. Add 150 $\mu$ L Substrate Solution into each well. Cover and Incubate for 15 minutes at 37°C.
7. Add 100 $\mu$ L Stop Solution to each well. Mix well.
8. Read the Optical Density (O.D.) at 450nm using a microtiter plate reader within 30 minutes.

NOTE: READ THE ABSORBANCES IMMEDIATELY AFTER COMPLETING THE ASSAY.

### **CALCULATION OF RESULTS**

Examine data for acceptance consistency with quality control guidelines. Aberrant values may be rejected.

Refer to the sample data and calculations,

- For each standard, control and unknown sample, the optical density values are averaged (if there is a duplicate).
- On millimeter paper using the ordinate for the optical density (or the %B/B<sub>0</sub>) and the abscissa for the standard concentrations ( $\mu$ g/dL), a smooth standard curve is plotted.
- The values of the control and of unknown samples are read directly from the standard curve.

## TYPICAL DATA

### EXAMPLE

Results of a typical standard run are shown below:

**TABLE I**

Standard (µg/dL)	O.D. (450nm)	Concentration (µg/dL)
0	2.645	
3	2.200	
6	1.777	
9	1.077	
12	0.696	
18	0.454	
Serum	2.179	2.717
Serum	1.117	9.378
Serum	0.590	14.645
Etc...	...	...

## PERFORMANCE CHARACTERISTICS

1. **SENSITIVITY:** Sensitivity is defined as the minimum concentration of T4 that can be statistically distinguished from standard 0 µg/dL. This method will reliably detect T4 concentrations as low as 0.27 µg/dL
2. **PRECISION:**
  - a) **Intra-assay Precision:** To determine within-run precision, 3 different samples of known concentrations were assayed by using 10 replicates in one assay:

Parameters	Samples		
	1	2	3
Number of determinations (N)	10	10	10
Mean (µg/dL)	6.936	2.767	1.982
Standard deviation (µg/dL)	0.233	0.084	0.080
Coefficient of variation (%)	3.36	3.03	4.03

- b) **Inter-assay Precision:** To determine between-run precision, 3 different samples of known concentration were assayed by using replicates on 20 different assays.

Parameters	Samples		
	1	2	3
Number of determinations (N)	20	20	20
Mean (µg/dL)	6.679	3.14	2.068
Standard deviation (µg/dL)	0.571	0.276	0.197
Coefficient of variation (%)	8.56	8.78	9.54

3. **ACCURACY:** The data obtained are indicated below:

Samples	Expected Value (µg/dL)	Observed value (µg/dL)
1	2.5-8.8	3.34
2	5.5-12.7	8.96
3	8.5-18.6	13.42

4. **RECOVERY:** The recovery of T4 spiked to 3 different levels in different serum throughout the range of the assay was evaluated.

Sample type	Average recovery (%)	Range (%)
Normal Serum #1	93	82-107
Normal Serum #2	107	103-113

5. **SPECIFICITY:** The following hormones and chemicals were tested for cross-reactivity:

HORMONE TESTED	CONCENTRATION	PRODUCED COLOR INTENSITY EQUIVALENT TO T4 (µg/dL)
Thyroxine(T4)	3 ug/dL	3
	6 ug/dL	6
	9 ug/dL	9
	18 ug/dL	18
Triiodo-L-Thyronine	20 ng/mL	0
	10 ng/mL	0
	4 ng/mL	0
Methimazole	1,000 ng/mL	0
	50,000 ng/mL	0
	500,000 ng/mL	0
Phenylbutazone	10,000 ng/mL	0
	50,000 ng/mL	0
	1,000,000 ng/mL	0
6-n-Propyl-2-Thiouracil	10,000 ng/mL	0
	100,000 ng/mL	0
	250,000 ng/mL	0
Sodium Salicylate	5,000 ng/mL	0
	500,000 ng/mL	0
	1,000,000 ng/mL	0
Diphenylhydantoin	1,000 ng/mL	0
	10,000 ng/mL	0
Triiodothyroacetic Acid	5 ng/mL	0
	10 ng/mL	0
	100 ng/mL	0
Diiodo-l-thyronine	1,000 ng/mL	0
	10,000 ng/mL	0
	50,000 ng/mL	0

## **QUALITY CONTROL**

Good laboratory practice requires that quality control specimens be run with each calibration curve to check the assay performance. Commercial controls are suitable for this purpose. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure adequate performance.

## **REFERENCES**

1. Bowers, C.Y., Chally, C., Gual, C., et al. *Biochem. Biophys. R.*, 39, 353 (1970).
2. Fleisher, N., Burgus, R., Vale, W., et al. *J. Clin. Endocr.*, 31, 109 (1970).
3. Anderson, M.S., Bowers, C.Y., Kastin, A.J., et al. *N.E.J. Med.*, 285, 1279 (1971).
4. Snyder, R.J. and R.D. Utiger, *J. Clin. Inv.*, 51, 2077 (1972).
5. Mitsuma, T., Colucci, J. Shenkman, L., et al. *Biochem. Biophys. R.* 46, 2107 (1972).
6. Chopra, I.J. *J. Clin. Endocr.*, 35, 938 (1972).
7. Stein, R.B., and L. Price, *J. Clin. Endocr. and Metab.*, 34, 225 (1972).