

Human T3 ELISA Kit

For the quantitative *in vitro* determination of Human Triiodothyronine (T3) concentrations in human serum samples

Catalogue Number: EL10006

96 tests

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



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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 T3 (Triiodothyronine) in small volumes of serum (50 μ L 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. When required, they are released into the bloodstream where they bind largely to specific binding proteins, which act as a mechanism of transport. The two major proteins are Thyroxine Binding Globulin (TBG) and Thyroxine Binding Pre-Albumin (TBPA). A small percentage of this circulating T4 and T3 remains unbound or free and this is the portion of the hormones believed to be physiologically active. Any abnormalities in the thyroid, pituitary or hypothalamus glands may alter the levels of these hormones in the bloodstream.

PRINCIPLE OF THE ASSAY

The ANOGEN coated well immunoenzymatic assay for the quantitative measurement of serum T3 utilizes a monoclonal anti-T3 and a T3-HRP conjugate. The assay asample and buffer are incubated together with anti-T3 antibody coated plate for sixty (60) minutes and washed. The diluted T3-HRP conjugate is then added to each well and incubated. After the incubation period, the wells are decanted and washed three times. The wells are then incubated with a substrate for the enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stopping solution is added to stop the reaction, which will then turn the solution yellow.

The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the T3 concentration since T3 from samples and T3-HRP conjugate compete for the anti-T3 antibody binding site. Since the number of sites is limited, as more sites are occupied by T3 from the sample, fewer sites are left to bind T3-HRP conjugate.

Standards of known T3 concentrations are run concurrently with the samples being assayed and a standard curve is plotted relating the intensity of the color (Optical Density, or O.D.) to the concentration of T3. The unknown T3 concentration in each sample is interpolated 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 T3 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.

96 tests	
1. MICROTITER PLATE (Part EL06-1) _____	96 wells
Pre-coated wells with anti-T3 monoclonal antibody immobilized into the well.	
2. ENZYME CONJUGATE (50X) (Part EL06-2) _____	1 mL
Concentrated T3-HRP conjugate in stabilizer solution.	
3. CONJUGATE DILUENT (Part EL06-3) _____	15 mL
PBS buffer pH 7.2 with preservative.	
4. T3 STANDARD – 20 ng/mL (Part EL06-4) _____	0.5 mL
Prepared with human T3 in normal human serum containing preservative.	
5. T3 STANDARD – 10 ng/mL (Part EL06-5) _____	0.5 mL
Prepared with human T3 in normal human serum containing preservative.	
6. T3 STANDARD – 4 ng/mL (Part EL06-6) _____	0.5 mL
Prepared with human T3 in normal human serum containing preservative.	
7. T3 STANDARD – 2 ng/mL (Part EL06-7) _____	0.5 mL
Prepared with human T3 in normal human serum containing preservative.	
8. T3 STANDARD – 1 ng/mL (Part EL06-8) _____	0.5 mL
Prepared with human T3 in normal human serum containing preservative.	
9. T3 STANDARD – 0.5 ng/mL (Part EL06-9) _____	0.5 mL
Prepared with human T3 in normal human serum containing preservative.	
10. T3 STANDARD – 0 ng/mL (Part EL06-10) _____	1 mL
Prepared with human T3 in normal human serum containing preservative.	
11. SUBSTRATE A (Part EL06-11) _____	11 mL
Buffered solution with H ₂ O ₂ .	
12. SUBSTRATE B (Part 30007) _____	11 mL
Buffered solution with TMB.	
13. WASH BUFFER (20X) (Part 30005) _____	60 mL
Concentrated solution of saline phosphate buffer. Dilute each bottle to one (1) liter with deionized or distilled water.	
14. STOP SOLUTION (Part EL30008) _____	14 mL
2 N Sulfuric Acid (H ₂ SO ₄). CAUTION: Caustic Material!	

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Precision pipettes (50 μ L) with disposable tips or a SMI pipette
2. 8 channels pipette (100 and 150 μ 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 T3 procedure. No additives or preservatives 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 (50X) with Conjugate Diluent in the proportion of 1 volume of Enzyme Conjugate (50X) in 50 volumes of Conjugate Diluent (Dilution 1/50). 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 (50X)	Volume (mL) Conjugate Diluent
24	0.06	3.0
48	0.12	6.0
72	0.18	9.0
96	0.24	12.0

Discard unused portion of diluted Enzyme Conjugate (1X) 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 $22 \pm 2^\circ\text{C}$ BEFORE ASSAYING.
 - ALL REAGENTS AND PATIENT SAMPLES SHOULD BE MIXED BY SWIRLING OR GENTLY VORTEXING. DO NOT INDUCE FOAMING.
1. Prepare Wash Buffer and T3 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
1A, 2A	Standard 1 – 20 ng/mL (S1)	1E, 2E	Standard 5 - 1 ng/mL (S5)
1B, 2B	Standard 2 – 10 ng/mL (S2)	1F, 2F	Standard 6 – 0.5 ng/mL (S6)
1C, 2C	Standard 3 – 4 ng/mL (S3)	1G, 2G	Standard 7 - 0 ng/mL (S7)
1D, 2D	Standard 4 – 2 ng/mL (S4)	1H-12H	T3 samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	3	11	19	27	35	43	51	59	67	75
B	S2	S2	4	12	20	28	36	44	52	60	68	76
C	S3	S3	5	13	21	29	37	45	53	61	69	77
D	S4	S4	6	14	22	30	38	46	54	62	70	78
E	S5	S5	7	15	23	31	39	47	55	63	71	79
F	S6	S6	8	16	24	32	40	48	56	64	72	80
G	S7	S7	9	17	25	33	41	49	57	65	73	81
H	1	2	10	18	26	34	42	50	58	66	74	82

2. Add 50µL of T3 Standard or Sample to the appropriate wells.

3. Add 100 μ L of T3 HRP conjugate 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 μ L/well/wash (range: 350-400 μ 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 μ L Substrate Solution into each well. Cover and Incubate for 15 minutes at 37°C.
7. Add 100 μ 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/B₀) and the abscissa for the standard concentrations (ng/mL), 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:

Standard (ng/mL)	O.D. (450nm)	Concentration (ng/mL)
0	2.673	
0.5	2.453	
1.0	2.362	
2.0	2.231	
4.0	1.833	
10.0	1.304	
20.0	0.900	
Serum	2.407	1.103
Serum	2.124	2.337
Serum	1.923	3.372
Etc...

PERFORMANCE CHARACTERISTICS

- SENSITIVITY:** The sensitivity of the assay, as defined by the amount of T3 standard that will reduce the maximal binding ($B_0 = 100\%$ of the Standard 0) by 5% was calculated to be 0.32 ng/mL.
- PRECISION:**
 - Intra-assay Precision:** To determine within-run precision, 3 different samples of known concentration were assayed by using 10 replicates in one assay.

Parameters	Samples		
	1	2	3
Number of determinations (N)	10	10	10
Mean (ng/mL)	3.72	2.189	1.635
Standard deviation (ng/mL)	0.113	0.117	0.089
Coefficient of variation (%)	3.04	5.34	5.44

- 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 (ng/mL)	4.062	2.066	1.494
Standard deviation (ng/mL)	0.167	0.119	0.09
Coefficient of variation (%)	4.11	5.76	6.02

3. **RECOVERY:** The recovery of T3 was spiked to 3 different levels in different serum throughout the range of the assay and was evaluated:

Sample Type	Average Recovery (%)	Range (%)
Normal Serum #1	87	78-102
Normal Serum #2	98.3	70-120

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

HORMONE TESTED	CONCENTRATION	PRODUCED COLOR INTENSITY EQUIVALENT TO T3 (ng/mL)
Triiodo-L-Thyronine	1 ng/mL	1
	2 ng/mL	2
	4 ng/mL	4
	10 ng/mL	10
Thyroxine(T4)	9 ug/dL	1.774
	12 ug/dL	2.161
	18 ug/dL	2.942
Methimazole	1,000 ng/mL	0.629
	50,000 ng/mL	0.635
	500,000 ng/mL	0.731
Phenylbutazone	10,000 ng/mL	0
	50,000 ng/mL	0.377
	1,000,000 ng/mL	0.938
6-n-Propyl-2-Thiouracil	10,000 ng/mL	0
	100,000 ng/mL	0.059
	250,000 ng/mL	0.315
Sodium Salicylate	5,000 ng/mL	0.765
	500,000 ng/mL	1.404
	1,000,000 ng/mL	1.659
Diphenylhydantoin	1,000 ng/mL	0.048
	10,000 ng/mL	0.999
Triiodothyroacetic Acid	5 ng/mL	1.053
	10 ng/mL	1.306
	100 ng/mL	4.586
Diiodo-l-thyronine	1,000 ng/mL	9.193
	10,000 ng/mL	> 20
	50,000 ng/mL	> 20

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.

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