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PRODUCT DATA SHEET

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Free T_3 (Triiodothyronine) MICRO-ELISA Test Kit

Prod. No.: **T183**
Pkg. Size: **96 Tests**

Description

The MICRO-ELISA FREE TRIIODOTHYRONINE (fT_3) test is a solid phase competitive enzyme immunoassay (EIA) Diagnostic Kit for the in vitro quantitative determination of free triiodothyronine (T_3) concentration in human serum.

Summary and Explanation of the Test

The principal tests used in the laboratory evaluation of thyroid function are Total Thyroxin (T_4), Total Triiodothyronine (T_3), T-Uptake (T-Up), a calculated Free Thyroxin Index (FTI) and Thyroid Stimulating Hormone (TSH). The results of these tests are interrelated and help the clinician in making a diagnosis. Clinical hypothyroidism results from underproduction of thyroid hormones by the thyroid gland, consequently an abnormally low circulating T_4 and T_3 concentration in blood. Clinical hyperthyroidism results from excessive production of thyroid hormones and resulting elevation of T_4 and T_3 concentrations.

The manifestations of thyroid dysfunction can result from disease of the thyroid gland (PRIMARY hyperthyroidism or hypothyroidism), disease of the pituitary gland (SECONDARY hyperthyroidism or hypothyroidism) or disease of the hypothalamus (TERTIARY hyperthyroidism or hypothyroidism).

Thyroxin (3,5,3',5'-tetraiodo-l-thyronine, T_4) and Triiodothyronine (3,5,3'-triiodo-l-thyronine, T_3), are the hormones originating from the thyroid gland. T_4 and T_3 are responsible for regulating diverse biochemical processes throughout the body that are essential for protein synthesis, normal development, metabolic and neural activity.

T_4 is synthesized within the thyroid gland and secreted directly into the bloodstream. Approximately 30% of the circulating T_4 is enzymatically deiodinated at the 5' position in the peripheral tissues to yield T_3. The T_4 likely serves as a "prohormone" for T_3, which has a much greater metabolic activity.

T_4 and T_3 are transported through the peripheral blood stream largely bound to serum proteins. The major transport protein is Thyroxin Binding Globulin (TBG) which normally accounts for 80% of the bound hormone. The other thyroid hormone binding proteins are Thyroxin Binding Prealbumin and Albumin. Only about 0.3% of the total serum T_3 and only about 0.1% of the total serum T_4 are unbound and free to diffuse into tissue to exert their biological effects. When the level of TBG increases, the level of total T_4 and T_3 will increase to maintain the same level of unbound or free T_4 and T_3 in the bloodstream of an euthyroid individual.

Simply determining the total T_4 or total T_3 concentration fails to take into account the variations in TBG levels that affect the unbound hormone (free T_4 and free T_3) concentration. TBG levels can vary for reasons incidental to the patient's thyroid status such as the presence of certain drugs, steroid hormones, pregnancy, and various non-thyroidal diseases. The Thyroid Uptake (T-Up) test is an indirect measurement of empty binding sites for T_4 on the TBG molecule (unsaturated TBG) in the patient specimen. The number obtained from the multiplication of the Total T_4 concentration by the Thyroid Uptake value is called a Free Thyroxin Index (FTI). The FTI correlates more closely with Free T_4 (the metabolic active fraction) concentration than does the total T_4 concentration alone. The FTI is therefore a better method of monitoring thyroid function and diagnosing thyroid illness than is a Total T_4 determination alone.

Diseases of the thyroid gland can result in clinical signs of thyroid dysfunction. Primary hypothyroidism results in underproduction of T_4 by the thyroid gland and consequently an abnormally low circulating T_4 concentration in the blood. Primary hyperthyroidism leads to excessive thyroid production of T_4 and a resulting elevated T_4 concentration.

The determination of total serum T_3 is used in the differential diagnosis of thyroid disease, particularly hyperthyroidism. In most hyperthyroid patients, both serum T_3 and T_4 are elevated. However, approximately 5-10% of hyperthyroid patients have elevated T_3 concentrations but normal serum T_4, a condition known as T_3-thyrotoxicosis. Such clinical conditions make it vital to establish that serum T_3 is normal before excluding the diagnosis of hyperthyroidism. Serum T_3 level is also an excellent indicator for the ability of the thyroid to respond to both stimulatory and suppressive tests.

The thyroid gland function is regulated by the level of Thyroid Stimulating Hormone (TSH) which is produced and secreted by the pituitary gland. TSH is produced by the anterior lobe of the pituitary gland and acts on the thyroid gland to release thyroid hormones. The release of TSH from the pituitary is regulated by the hypothalamus when it secretes TRH (thyrotropin releasing hormone).

In an euthyroid individual, the levels of thyroid hormones in the blood are inversely related to the levels of TSH and TRH. When the levels of thyroid hormones rise, the levels of TRH and TSH fall; and when the levels of thyroid hormones fall, the levels of TRH and TSH rise. In the event of failure of the thyroid gland, the levels of thyroid hormones fall and the negative feedback results in an elevated level of TSH in the blood. Elevated levels of TSH are thus useful in the diagnosis of primary hypothyroidism. Conversely, in the case of primary hyperthyroidism, the elevated levels of thyroid hormones will result in decreased levels of TSH.

When there is a failure of the pituitary or the hypothalamus (secondary or tertiary hypothyroidism), the level of TSH is decreased in the presence of low levels of thyroid hormones. In secondary or tertiary hyperthyroidism, the level of TSH is increased in the presence of high levels of thyroid hormones.

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	T_4	T_3	T-UP	FTI	TSH
Euthyroid	N	N	N	N	N
Pregnant Euthyroid	I	I	D	N	N
1^o Hyperthyroidism	I	I	I	I	D
2^o or 3^o Hyperthyroidism	I	I	I	I	I
T_3 Thyrotoxicosis	N	I	N	N or I	N or D
1^o Hypothyroidism	D	D	D	D	I
2^o or 3^o Hypothyroidism	D	D	D	D	D

N = Normal D = Decreased I = Increased

Principle of the Procedure

The MICRO-EIA Free T_3 test is based on the principle of a solid phase competitive enzyme immunoassay (EIA). The assay system utilizes a highly specific T_3 polyclonal antibody bound to a polystyrene well and an enzyme-labeled analyte. Test sample and T_3-enzyme conjugate are added to each antibody coated well. During a 60 minute incubation, free T_3 in the patient's sera competes with the T_3-enzyme conjugate for binding sites on the coated wells. The number of binding sites on the well are limited; as more of them are occupied by the free T_3 from the sample, less of the T_3 enzyme conjugate can bind. The amount of free T_3 in the patient serum is inversely proportional to the amount of T_3-enzyme conjugate bound to the well. After a short incubation, the wells are washed to remove any unbound T_3-enzyme conjugate. An enzyme substrate-chromogen (hydrogen peroxide, **H_2O_2**, and tetramethylbenzidine, **TMB**) is added to the well and incubated for 15 minutes at room temperature, resulting in the development of a blue color. The addition of 1.0 N H_2SO_4 stops the reaction and converts the color to yellow and increases the absorbance by a factor of approximately 3. The intensity of the yellow color is inversely proportional to the concentration of free T_3 in the sample. The concentration of free T_3 in the patient sample is interpolated from a standard curve relating the absorbance, measured spectrophotometrically at 450 nm, of each calibrator to the concentration of free T_3.

Reagents

Components in Each 96 Test

Micro-ELISA Ft_3 Diagnostic Kit

1. 96 wells, T_3 **ANTIBODY COATED WELLS**: Coated with anti T_3 (sheep polyclonal); contained in a pack with silica gel desiccant.
2. 1 bottle, 1.0 ml, Free T_3-**ENZYME CONJUGATE**: T_3-labeled horseradish peroxidase in a buffered protein solution; contains a preservative.
3. 1 bottle, 11 ml, **ASSAY BUFFER**: Buffered protein solution; contains a preservative.
4. 1 vial, 1.0 ml, Free T_3 **SERUM STANDARD, 0 pg/ml**: Human serum; contains a preservative.
5. 5 vials, 1.0 ml, Free T_3 **SERUM STANDARDS, 1.0, , 4.0, 8.0 AND 16.0 pg/ml**: Human serum with added T_3; contains a preservative. **NOTE: Exact value of Standards are lot specific and are listed on the vial label.**
6. 1 bottle, 20 ml, **WASH BUFFER CONCENTRATE (50X)**: Buffered detergent solution; contains a preservative. Dilute bottle to 1000 ml with deionized water.
7. 1 bottle, 12 ml, **SUBSTRATE CHROMOGEN**: Buffered hydrogen peroxide and 3,3',5,5' tetramethylbenzidine (TMB) solution.
8. 1 bottle, 12 ml, **STOP SOLUTION** 1 N H_2SO_4.

Additional Materials Required

Disposable tip precision pipettes 0.050, and 0.1 ml microtiter plate reader.
Absorbent paper.
Distilled or deionized water.

Storage and Stability

Store all components at 2° 8°C when not in use. The wells, substrate/chromogen, wash buffer and stop solution may be stored at ambient temperature. Expiration date printed on the kit indicates limits of stability.

The T_3 ANTIBODY COATED WELLS are supplied in a resealable bag containing a desiccant and must be stored with the bag sealed to protect from moisture. Wells can be stored at 2°- 30°C.

Chemical or Physical Indications of Instability

Alterations in the physical appearance of reagents, or results consistently outside the acceptable limits for control sera, may be due to reagent contamination or deterioration.

Instruments

Performance of the Free T_3 test requires use of a precision microtiter plate reader at a wavelength of 450 ± 20 nm:

Specimen Collection and Preparation

Serum samples are used in the MICRO-EIA Free T_3 Diagnostic Kit procedure. No special preparation of the patient is necessary; fasting is not required. Repeated freezing and thawing of specimens should be avoided. No additives or preservatives are necessary.

STORAGE: Specimens may be stored in a tightly stoppered tube at 2°- 8°C for two days. If the serum is not assayed within 2 days, store frozen (- 20°C) in a tightly sealed tube for up to 3 weeks. Specimens should be allowed to come to room temperature and should be mixed thoroughly by gentle inversion before assaying.

Do not use grossly lipemic specimens. Moderately lipemic, hemolyzed and icteric specimens should not interfere with the assay.

Micro-ELISA Free T_3 Procedure

Reagent Preparation

WORKING CONJUGATE. Dilute only enough conjugate for a single assay run. Dilute the conjugate 1:11 with the assay buffer. Prepare 0.1 ml of working conjugate for each well. Label the bottle and store at 2°-8°C. Expiration time is 24 hours.

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Dilute the entire contents of the WASH BUFFER to 1,000 ml with deionized water. Expiration date is the same as the concentrate. Store at 2°-8°C.

Preliminary Comments and Precautions

Patient sample may contain pathogens: treat all samples as potentially infectious.

CAUTION: Source material used to prepare Standards was derived from human material. The material was tested using FDA-approved methods and found non-reactive for Hepatitis B Surface Antigen (HBsAg) by ELISA and non-reactive for HIV by ELISA. No known test method can offer total assurance that infectious agents are absent. HANDLE THESE REAGENTS AS IF THEY ARE POTENTIALLY INFECTIOUS. Information on handling human serum is provided in the CDC/NIH manual "Bio-safety in Microbiological and Biomedical Laboratories" (1984).

Procedural Notes

1. When pipeting reagents, maintain a consistent order of addition from well to well. This will ensure equal incubation times for all wells. Carry out each addition step without pausing. The timing sequence in the addition of each reagent should be the same for all wells.
2. Samples should be pipetted to the bottom of the coated wells.
3. SINGLE POINT CALIBRATION USING A STORED CURVE.

The following optional procedure may be used:

a. For each new kit lot, run one complete standard curve. This standard curve may be used for up to 30 days.

b. For all subsequent sample runs, only the 0 pg/ml standard and controls need to be run with the patient serum samples as described in the Assay Procedure.

c. See the Results Section to calculate patient sample Free T_3 values using the single point calibration method.

CAUTION: If control values deviate from their established range, then the assay should be re-calibrated with a new standard curve.

NOTE: It is important in using a single point calibration that:

1. The assay procedure should be the same from run to run.
2. The same spectrophotometer or instrument should be used.
3. The spectrophotometer and all pipettes should be calibrated for accuracy and precision.
4. All test kit components used in an assay must be of the same master lot number. Materials should not be used after the expiration date shown on the package label. Components and test specimens should be at room temperature (18°- 30°C) before testing.

Test Procedure

PREPARE WORKING CONJUGATE: TO AN ALIQUOT OF ASSAY BUFFER (**0.1 ml** for each WELL) ADD THE REQUIRED VOLUME OF STOCK CONJUGATE (**10µl** for each WELL). MIX GENTLY.

1. Pipet **50 µl** of Free T_3 standards into the appropriate well. (Only the 0 pg/ml standard need be run if using the previously stored curve).
2. Pipet **50 µl** of each control and patient serum into the appropriate well.
3. Pipet **100 µl** (0.1 ml) of **fresh working** Free T_3-enzyme conjugate into all wells and mix gently.
4. Incubate at room temperature (18°- 30°C) for **60 minutes** ± 5 minutes.
5. Decant or aspirate and discard liquid contents of all wells.
6. Fill each well with diluted WASH BUFFER. Decant or aspirate liquid contents of all wells. Do not use tap water.

WARNING: WASHING THE WELLS IS OF CRITICAL IMPORTANCE. Fill the wells to overflowing, you CANNOT cause any carryover between wells. You CANNOT over wash the wells. Completely decant or aspirate all of the liquid out of the wells. SLAP the inverted wells on a FRESH clean piece of absorbent paper AFTER EACH WASH. YOU CANNOT SLAP TOO HARD, REMOVE ALL OF THE LIQUID FROM THE WELLS.

7. Repeat step 6 twice more (for a **total of 3 washes**). Tap wells free of any liquid or aspirate thoroughly.
8. Pipet or dispense **100 µl** (0.1 ml) of SUBSTRATE / CHROMOGEN REAGENT into each well.
9. Mix thoroughly and incubate **15 minutes** at room temperature (18°-30°C).
10. Pipet or dispense **100 µl** (0.1 ml) of STOPPING REAGENT into each well and mix thoroughly.
11. Read the absorbance of each well at **450 ± 20 nm** against water.

Calculation of Results

1. Calculate the $\%A/A_0$ value for each standard, control and sample.

$$\%A/A_0 = \frac{\mathbf{A}}{\mathbf{A_0}} \quad X\,(100\%)$$

A = the average absorbance value for the standard, controls and patient samples.

A_0 = the average absorbance value for the 0 pg/ml standard.

2. Construct a standard curve by plotting the $\%A/A_0$ value for the thyroxin standards (vertical axis) versus the free T_3 standard concentration (horizontal axis) on the logit-log graph paper supplied.
3. Draw the best straight line through the points.
4. Interpolate the control and patient sample values from each $\%A/A_0$ value obtained.
5. Save the calibration curve for use in subsequent runs using only single point calibration (0 pg/ml).

Optional calculation method for use with single point calibration and stored standard calibration curve.

a. For single point calibration runs, calculate $\%A/A_0$ values for samples and controls using the absorbance of the 0 pg/ml standard that was run with them.

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b. Using the original standard calibration curve, interpolate the control and patient sample values from each $\%A/A_0$ value obtained in the run.

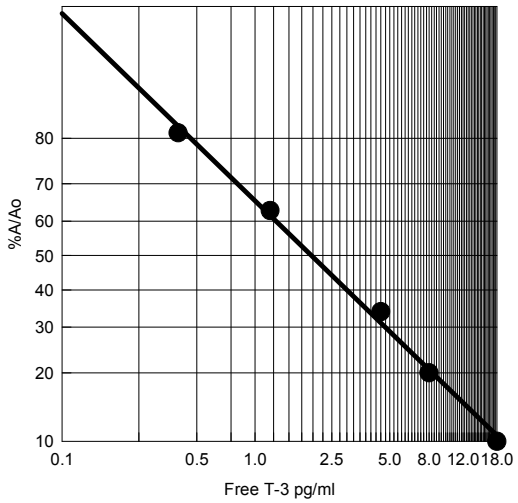
NOTE: For automated data reduction, use a log/logit date transformation of A/A_0 vs. Free T_3 concentration.

EXAMPLE DATA

Specimen I.D		A_{450}	$\%A/A_0$	Calculated Value
STANDARD	0 pg/ml	2.063	100%	
		2.138		
STANDARD	0.6 pg/ml	1.815	88%	
		1.866		
STANDARD	1.3 pg/ml	1.546	75%	
		1.595		
STANDARD	4.0 pg/ml	0.983	46%	
		0.968		
STANDARD	8.0 pg/ml	0.558	27%	
		0.589		
STANDARD	16.0 pg/ml	0.335	16%	
		0.348		
PATIENT	1	1.787	86%	0.7 pg/ml
		1.836		
PATIENT	2	1.231	59%	2.7 pg/ml
		1.226		
PATIENT	3	0.763	37%	5.7 pg/ml
		0.786		

The range of this assay is 0 – 16.5 pg/ml. For specimen with Free T_3 concentrations beyond the standard curve (18 pg/ml), repeat the test by diluting the specimen with the 0 pg/ml Standard. To obtain the final concentration, multiply the concentration of the diluted sample by the dilution factor.

MICRO-EIA Free T-3



Limitations of the Procedure

As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

In patients receiving drug therapy, the equilibrium between Free T_3 and TBG bound T_3 may be affected.[4] Heparin therapy is known to increase the concentration of non-esterified fatty acids which may displace T_3 on serum binding proteins and thus cause an elevation of Free T_3 values. Phenytoin, salicylate and other drugs can also interfere with the binding of T_3 to TBG[5] in much the same way. One must be cautious when interpreting Free T_3 results from patient being treated with heparin or other drugs which affect binding of T_3 to serum proteins.

TBG concentrations have been reportedly altered by increased estrogens, anabolic steroids, androgens, glucocorticoid and pregnancy [6,7,8] Major illness, surgical stress, genetic deficiency and hepatitis can also affect TBG concentrations, with possible consequences on fT_3 levels.

Familial dysalbuminemic hyperthyroxinemia[9], auto-antibodies to T_3[10] and analbuminemia[11] can result in elevations of Total T_3 but should not affect fT_3 levels unless the patients are receiving T_3 treatment.[10]

Alterations in the concentration of serum binding proteins will generally result in corresponding change in Total T_3 concentrations while the physiologically active Free T_3 level remains largely unchanged in a euthyroid individual. Therefore, determination of Free T_3 concentration may provide a more accurate assessment of thyroid status than Total T_3 measurement. Elevated Free T_3 concentrations are indicative of hyperthyroidism and low levels are indicative of hypothyroidism.

Improper handling of patient samples may cause spurious results. Avoid using old or mistreated serum specimens. Sample degradation may cause inaccurate fT_3 determinations. Patient specimens should be assayed as soon as possible. Severely hemolytic, lipemic or icteric samples may result in poor precision or inaccurate values. Results obtained from these types should be viewed with caution.

The wash procedure (steps 6 8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbances. The use of tap water for washing could result in a higher background absorbance.

FINAL REACTION STABILITY: The spectrophotometric measurement should be made within 30 minutes after the addition of the STOPPING REAGENT solution.

Quality Control

Good laboratory practice requires that quality control specimens be run with each patient sample run to check the assay performance. Three controls with normal, low and elevated values should be used. Pooled human serum or commercially available control sera are suitable. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

Do not mix or interchange reagent from kits with different lot numbers. Pool and mix reagents from different bottles before use.

Do not use reagents beyond the expiration date printed on each vial or bottle.

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Free T_3 Expected Values

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for Free T_3 were established by the manufacturer and may be used as an initial guideline:

Hypothyroid:	less than 1.4 pg/ml
Euthyroid:	1.4 to 4.2 pg/ml
Hyperthyroid:	greater than 4.2 pg/ml

Performance Characteristics of the Test

Assay Specificity

Specificity of this test system was proven by determining the interference of the following cross-reactants in the Free T_3 assay. Results are expressed as the ratio of free T_3 concentration to the concentration of the cross-reactant that will displace 50% of the T_3 enzyme conjugate.

Cross-Reactant	% Cross-Reactivity
l-Triiodothyronine (T_3)	100
l-Thyroxin	<0.01
Diiodothyronine	< 0.01
Diiodotyrosine	< 0.01
Iodotyrosine	< 0.01

Assay Sensitivity

The sensitivity of this assay is defined as the smallest single value which can be distinguished from the zero calibrator. This value was calculated from the mean + two standard deviations for twenty one replicates at the zero concentration. The calculated sensitivity is < 0.05 pg/ml.

Assay Reproducibility

Intra assay reproducibility was determined by measurement of 10 replicates of three serum pools in a single run.

	Mean fT_3 (pg/ml)	SD	%CV
Serum A	1.42	0.15	10.3
Serum B	2.51	0.13	5.1
Serum C	5.11	0.20	4.0

The interassay reproducibility was determined by duplicate measurement of three serum pools in twelve separate runs.

	Mean fT_3 (pg/ml)	SD	%CV
Serum A	1.37	0.13	9.8
Serum B	2.47	0.15	6.0
Serum C	4.97	0.17	3.5

Correlation with Radioimmunoassay

A total of 54 serum samples were run in the MICRO-EIA fT_3 procedure and in a commercially available RIA procedure.

	Slope	Y Intercept	Correlation Coefficient
n= 54	0.97	0.14	0.976

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Warranty

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