Access
Immunoassay Systems

FREE T4

REF 33880 **FOR USE WITH TEST NAME: FRT4**

Intended Use

The Access Free T4 assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of free thyroxine levels in human serum and plasma (heparin) using the Access Immunoassay Systems.

Summary and Explanation

The hypothalamic-pituitary-thyroid axis controls thyroid hormone synthesis, release, and action. Thyrotropin-releasing hormone (TRH) secreted from the hypothalamus stimulates the synthesis and release of thyrotropin or thyroid-stimulating hormone (TSH). TSH, in turn, stimulates the synthesis, storage, secretion, and metabolism of thyroxine (T4) and triiodothyronine (T3). Both free and bound forms of T4 and T3 are present in the blood. More than 99% of the T4 and T3 circulate in the blood bound to carrier proteins, leaving less than 1% unbound. It is this level of unbound or free hormone that correlates with the functional thyroid state in most individuals.[1,2]

Free T4 and free T3 regulate normal growth and development by maintaining body temperature and stimulating calorigenesis. In addition, free T4 and free T3 affect all aspects of carbohydrate metabolism as well as certain areas of lipid and vitamin metabolism. Fetal and neonatal development also require thyroid hormones.[1,2]

Clearly elevated free T4 levels support the clinical findings of a diagnosis of hyperthyroidism while clearly low free T4 levels coupled with appropriate clinical findings, can establish a diagnosis of hypothyroidism. Measurement of free T4 levels along with other thyroid tests and clinical findings can establish borderline hyperthyroid and hypothyroid diagnoses.[3]

Equilibrium dialysis RIA is considered the reference method for measuring free T4 because it allows for the separation of free T4 from protein bound T4 before direct measurement of the free T4.[2] However, this method is cumbersome, technically demanding, and not suited to routine clinical laboratory use. More recently, radioimmunoassays and enzyme immunoassays have been developed for measuring free T4. These assays employ various combinations of analog or non-analog tracers and one-step or two-step incubation procedures.

Principles of the Procedure

The Access Free T4 assay is a two-step enzyme immunoassay. Monoclonal anti-Thyroxine (T4) antibody coupled to biotin, sample, buffered protein solution, and streptavidin-coated solid phase are added to the reaction vessel. During this first incubation the anti-T4 antibody coupled to biotin binds to the solid phase and the free T4 in the sample. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Next, buffered protein solution and triiodothyronine (T3)-alkaline phosphatase conjugate are added to the reaction vessel. The T3-alkaline phosphatase conjugate binds to the vacant anti-T4 antibody binding sites. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of free T4 in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Product Information

Access Free T4 Reagent Pack

Cat. No. 33880: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C for 28 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent is damaged (i.e. broken elastomer), discard the pack.
- All antisera are polyclonal unless otherwise indicated.

R1a:	Dynabeads** paramagnetic particles coated with streptavidin in a TRIS buffer with protein (aves), surfactant, 0.125% NaN_3, and 0.125% ProClin*** 300.
R1b:	TRIS buffered saline with protein (aves), surfactant, < 0.1% NaN_3, and 0.1% ProClin 300.
R1c:	TRIS buffered saline with protein (aves), surfactant, 0.125% NaN_3, and 0.125% ProClin 300.
R1d:	Triiodothyronine-alkaline phosphatase (bovine) conjugate in a TRIS buffer with protein (aves), surfactant, < 0.1% NaN_3, and 0.1% ProClin 300.
R1e:	Mouse monoclonal anti-Thyroxine (T4) coupled to biotin in a TRIS buffer with protein (aves and murine), surfactant, 0.125% NaN_3, and 0.125% ProClin 300.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[4]
- Xi. Irritant: 0.125% ProClin 300.

R 43: May cause sensitization by skin contact.

S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- Xn. Harmful: 0.125% Sodium Azide (NaN_3).

R 22: Harmful if swallowed.

S 28: After contact with skin, wash immediately with plenty of water.

- The Material Safety Data Sheet (MSDS) is available upon request.

Specimen Collection and Preparation

1. Serum and plasma (heparin) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples:[5]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
   - Thaw samples only once.
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter has been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot to lot.

Materials Provided

R1 Access Free T4 Reagent Packs

Materials Required But Not Provided

1. Access Free T4 Calibrators
   Provided at zero and approximately 0.5, 1.0, 2.0, 3.0 and 6.0 ng/dL (6.4, 12.9, 25.7, 38.6 and 77.2 pmol/L).
   Cat. No. 33885
2. Quality Control (QC) materials: commercial control material.
3. Access Substrate
   Cat. No. 81906
4. **Access, Access 2, SYNCHRON LXi:**
   Access Wash Buffer II, Cat. No. A16792
   UniCel DxI:
   UniCel DxI Wash Buffer II, Cat. No. A16793

Procedural Comments

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use thirty (30) µL of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. The system default unit of measure for sample results is ng/dL. To change sample reporting units to the International System of Units (SI units), pmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply ng/dL by multiplication factor 12.87.

Procedure

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

Calibration Details

An active calibration curve is required for all tests. For the Access Free T4 assay, calibration is required every 28 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Quality Control

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[6] Include commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

Results

Patient test results are determined automatically by the system software using a weighted four parameter logistic curve (4PLC) math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

Limitations of the Procedure

1. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.25–6.0 ng/dL [3.2–77.2 pmol/L]).
   - If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e., < 0.25 ng/dL [< 3.2 pmol/L]).
   - If a sample contains more than the stated value of the highest Access Free T4 Calibrator (S5), report the result as greater than that value (i.e., > 6.0 ng/dL [> 77.2 pmol/L]). **SAMPLES CANNOT BE DILUTED FOR FREE T4 DETERMINATIONS.**
2. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[7,8]

   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. The Access Free T4 results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information.
4. Samples containing thyroxine autoantibodies can be assayed in two step procedures such as the Access Free T4 assay without significant interference.[2]
5. Non-thyrometabolic disorders may cause abnormal free T4 levels. Anticonvulsant drug therapy (particularly phenytoin) may result in decreased free T4 levels due to an increased hepatic metabolism, and secondarily to displacement of hormone from binding sites.[2,9,10] Anti-inflammatory drugs such as salicylate and phenylbutazone also compete for hormone binding sites, but their effect on free T4 levels has not been clearly defined.[2,11] Patients on heparin therapy may have elevated free T4 levels due to release of non esterified fatty acids, which can alter the relationship between free and bound hormones.[10] Determination of thyroid status in patients with non-thyroidal illness (NTI) should be interpreted with caution. [2,12] In rare conditions, such as Familial Dysalbuminemic Hyperthyroxinemia (FDH),

direct free hormone assays may yield erroneous results due to the extreme variations in the albumin-binding capacity for T4.

The following substances were added to euthyroid serum samples. When tested in the Free T4 assay, the observed mean percent changes in Free T4 values were as follows:

Substance	Amount Added (mg/dL)	% Change
Aspirin	60	+6.4
Sodium Salicylate	50	+9.1
Phenylbutazone	7.5	+8.48
Thiouracil	5.0	-0.6
Phenytoin	5.0	+8.0
Methimizole	0.4	+0.9

6. This assay is not significantly influenced by the presence of thyroxine hormone binding proteins. In one study, the zero calibrator, which contains normal levels of protein, was spiked with the following human source thyroxine hormone binding proteins. Below is the observed change to the assay signal response.

Substance	Amount Added	% Change
Albumin	10.0 g/dL	-7.2
Thyroxine binding globulin (TBG)	160 µg/mL	-5.6
Prealbumin	600 µg/mL	-1.3

Expected Values

1. Sera samples were obtained from a minimum of 150 males and 150 females ranging in age from 18–60 years old. The samples were collected from the east, west and central United States. Following the guidance of both the National Academy of Clinical Biochemists (NACB) Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease[13] and the American Association of Clinical Endocrinologists,[14,15,16] the following screening criteria was utilized: TSH value 0.3–3.0 µIU/mL, no known personal or family history of thyroid disease or autoimmune disease and the absence of thyroid medication. After completing the Access TSH screen, 32 samples were excluded due to TSH values outside of the 0.3–3.0 µIU/mL range.

n	95% Reference Limit (ng/dL)	95% CI for Lower Limit (ng/dL)	95% CI for Upper Limit (ng/dL)
316	0.61–1.12	0.54–0.67	1.07–1.24

n	95% Reference Limit (pmol/L)	95% CI for Lower Limit (pmol/L)	95% CI for Upper Limit (pmol/L)
316	7.86–14.41	7.00–8.57	13.73–15.96

2. Sera samples were obtained from a minimum of 120 women in the first, second and third trimester of pregnancy.

Sample Type	n	95% Reference Limit (ng/dL)	90% CI for Lower Limit (ng/dL)	90% CI Upper Limit (ng/dL)
1st Trimester	131	0.52–1.10	0.47–0.57	1.08–1.27
2nd Trimester	120	0.45–0.99	0.40–0.48	0.80–1.08
3rd Trimester	121	0.48–0.95	0.45–0.51	0.83–1.23

Sample Type	n	95% Reference Limit (pmol/L)	90% CI for Lower Limit (pmol/L)	90% CI Upper Limit (pmol/L)
1st Trimester	131	6.67–14.12	6.00–7.31	13.86–16.28
2nd Trimester	120	5.79–12.70	5.19–6.14	10.24–13.86
3rd Trimester	121	6.11–12.20	5.77–6.62	10.68–15.79

3. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.
4. The Access Free T4 Assay has not been validated for dry blood spot samples.

Specific Performance Characteristics

Methods Comparison

A comparison of serum free T4 values using the Access Free T4 assay on the Access Immunoassay System and a commercially available immunoassay kit gave the following statistical data using Deming calculations:

Sample Type	n	Range of Observations (ng/dL)	Intercept (ng/dL)	Slope	Correlation Coefficient (r)
Serum	327	0.27–5.11	-0.275	1.16	0.940

A comparison of free T4 values obtained by assaying pairs of serum and plasma (heparin) samples using the Access Free T4 assay kit gave the following statistical data using Deming calculations:

Sample Type	n	Range of Observations (ng/dL)	Intercept (ng/dL)	Slope	Correlation Coefficient (r)
Serum vs. plasma (heparin)	51	0.61–1.06	0.033	0.962	0.980

Imprecision

This assay exhibits total imprecision of ≤ 10% CV at concentrations ≥ 0.61 ng/dL and < 0.06 ng/dL SD at concentrations < 0.61 ng/dL.

One study, using three commerically available human serum based control materials and one patient serum sample generating a minimum of 38 assays, 2 replicates per assay over a minimum of 19 days provided the following data, analyzed via analysis of variance (ANOVA).[17,18]

		Within Run		Between Run		Total	
Sample	Mean (ng/dL)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	0.46	0.02	4.40	0.04	8.08	0.04	9.20
2	0.76	0.02	2.12	0.03	4.47	0.04	4.95
3	2.04	0.06	2.74	0.07	3.34	0.09	4.32
4	4.27	0.08	1.82	0.20	4.71	0.22	5.05

Analytical Specificity/Interferences

Samples containing up to 10 mg/dL (171 µmol/L) bilirubin, lipemic samples containing the equivalent of 1800 mg/dL (20.32 mmol/L) triolein, and hemolyzed samples containing up to 1 g/dL (10 g/L) hemoglobin do not affect the concentration of free T4 assayed.

The following table describes the cross-reactivity of the antibody used in the Free T4 assay with substances which are similar in structure to T4. The antibody specificity is determined in a total thyroxine assay to avoid binding displacement of T4 by related compounds. The substances were added to an Access Free T4 calibrator pool and found to give the following results expressed by weight.

Substance	Analyte Added (µg/dL)	Cross-Reactivity (%)
L-T4	5	> 100
D-T4	10	71
L-T3	500	1.7
R-T3	100	23
Tetraiodothyroacetic Acid	25	4
D-T3	500	0.7
3,3′ L-T2	5000	< 0.1
3,5 L-T2	5000	< 0.1
3′5′ L-T2	5000	< 0.1
L-Tyrosine	5000	< 0.01
D-Tyrosine	5000	< 0.01
Monoiodotyrosine	5000	< 0.01
Diiodotyrosine	5000	< 0.01

Analytical Sensitivity

The lowest detectable level of free T4 distinguishable from zero (Access Free T4 Calibrator S0) with 95% confidence is 0.25 ng/dL. This value is determined by processing a complete six point calibration curve, controls, and 10 replicates of the zero calibrator in multiple assays. The analytical sensitivity value is calculated from the curve at the point that is two standard deviations from the mean measured zero calibrator signal.

Access
Immunoassay Systems

FREE T4 CALIBRATORS

REF 33885

Intended Use

The Access Free T4 Calibrators are intended to calibrate the Access Free T4 assay for the quantitative determination of free thyroxine levels in human serum and plasma (heparin) using the Access Immunoassay Systems.

Summary and Explanation

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e., assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

Traceability

The measurand (analyte) in the Access Free T4 Calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511.

The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Product Information

Access Free T4 Calibrators

Cat. No. 33885: S0–S5, 2.5 mL/vial

- Provided ready to use.
- Stable until the expiration date stated on the label when stored at -20°C or colder.
- Thaw at room temperature and mix contents by gently inverting before use. Avoid bubble formation.
- After thawing, calibrators are stable at room temperature for 2 hours.
- Return calibrators to -20°C after each use.
- Thaw calibrators no more than 5 times.
- Signs of possible deterioration are control values out of range.
- Refer to calibration card for exact concentrations.

S0:	Human serum with < 0.1% sodium azide, and 0.5% ProClin*** 300. Contains 0.0 ng/dL (0.0 pmol/L) thyroxine.
S1, S2, S3, S4, S5:	Free thyroxine in human serum at levels of approximately 0.5, 1.0, 2.0, 3.0 and 6.0 ng/dL (approximately 6.4, 12.9, 25.7, 38.6 and 77.2 pmol/L), respectively, with < 0.1% sodium azide, and 0.5% ProClin 300.
Calibration Card:	1

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their

origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.[19]
- Each serum/plasma pool used in the preparation of this product has been tested and found negative for the presence of fibrinogen.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[4]
- Xi. Irritant: 0.5% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

Procedure

Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Calibration Details

The Access Free T4 Calibrators are provided at six levels – zero and approximately 0.5, 1.0, 2.0, 3.0 and 6.0 ng/dL. Calibrators are prepared from purified thyroxine and human serum. Assay calibration data are valid up to 28 days.

Calibrators run in duplicate.

Limitations of the Procedure

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

References

1 Gornall, AG, Luxton, AW, Bhavnani, BR. Endocrine Disorders. In Applied biochemistry of clinical disorders. 1986; 305-318. Philadelphia, PA: J. B. Lippincott Co.

2 White, GH. Recent advances in routine thyroid function testing. CRC - Critical Reviews in Clinical Laboratory Sciences. 1987; 24: 315-362.

3 Watts, NB, Keffer, JH. The thyroid gland. In Practical Endocrine Diagnosis. 1982; 77-96. Philadelphia, PA: Lea & Febiger.

4 DHHS (NIOSH) Publication No. 78-127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available http://www.cdc.gov/niosh.

5 Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute.

6 Cembrowski GS, Carey RN. Laboratory quality management: QC ≒ QA. ASCP Press, Chicago, IL, 1989.

7 Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

8 Bjerner J, et al. Immunometric assay interference: incidence and prevention. Clin Chem 2002; 48: 613–621.

9 Liewendahl, K, Majuri, H, Helenius, T. Thyroid function tests in patients on long-term treatment with various anticonvulsant drugs. Clinical Endocrinology, 1978; 8: 187-191.

10 Wenzel, KW. Pharmacological interference with in vitro tests of thyroid function. Metabolis. 1981; 30(7): 717-732.

11 Wilke, TJ. Estimation of free thyroid hormone concentrations in the clinical laboratory. Clinical Chemistry. 1986; 32(4): 585-592.

12 Spencer, CA. Thyroid status: trends in testing – selective test use cuts cost. Clinical Chemistry News. November, 1989; 15(11): 9-14.

13 Demers, LM, Spencer, CA. Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. Clin Endocrinol (Oxf) 2003; 58(2): 138-140.

14 Lee, SL. When is the TSH normal? New criteria for diagnosis and management. 12th Annual Meeting of the American Association of Clinical Endocrinologists (AACE), May, 2003.

15 Haugen, BR. When isn't TSH normal and why? Clinical implications and causes. 12th Annual Meeting of the American Association of Clinical Endocrinologists (AACE), May, 2003.

16 Singer, PA. Now it's normal – now it's not: food for thought from a complex case. 12th Annual Meeting of the American Association of Clinical Endocrinologists (AACE), May, 2003.

17 Tentative Guideline - Internal quality control: Principles and definitions, C24-T. 1987. National Committee for Clinical Laboratory Standards.

18 Krouwer, JS, Rabinowitz, R. How to improve estimates of imprecision. Clinical Chemistry. 1984; 30: 290-292.

19 HHS Publication, 5th ed., December 2009. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/biosafety/publications/bmbl5/index.htm

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Printed in U.S.A.
Made in U.S.A. or Ireland
Revised January 2013