BECKMAN COULTER

ACCESS
Immunoassay Systems

Instructions For Use

Access Total T4 Thyroxine

REF 33800

FOR PROFESSIONAL USE ONLY

Rx Only

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

The Access Total T4 assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of total thyroxine (T4) levels in human serum and plasma 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). The essential features of thyroid hormone production and storage are: iodine uptake; iodination of tyrosine residues in the thyroglobulin molecule; coupling of the monoiodotyrosines (MIT) and diiodotyrosines (DIT) to form T4 and T3; storage of the thyronines as thyroglobulin in the thyroid gland; and release of thyroid hormones into the circulation. Once released into the circulation, most of the T4 and T3 are bound to carrier proteins. The greatest binding affinity for both hormones is to thyroxine-binding globulin (TBG) and, to a lesser extent, to prealbumin (TBPA). As a result, 99.97% of circulating T4 and 99.7% of circulating T3 bind leaving only small portions unbound.[1,2]

T4 and T3 regulate normal growth and development. They maintain body temperature, stimulate calorigenesis and 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]

Thyroxine is commonly measured in human serum as total T4, measuring both bound and free T4. It is used as a thyroid screening test alone or in conjunction with other thyroid tests. Measurement of total T4 gives a reliable reflection of clinical thyroid status in the absence of binding abnormalities. However, changes in binding proteins can occur which affect the level of total T4 but leave the level of unbound hormone unchanged.[2]

The clinical importance of total T4 determination is in the diagnosis and confirmation of thyroid disorders. Elevated levels of T4 occur in Graves' disease, subacute thyroiditis, toxic nodule, or secondary (pituitary) hyperthyroidism. Decreased levels occur in primary hypothyroid diseases such as Hashimoto's thyroiditis and neonatal hypothyroidism or secondary hypothyroidism due to defects at the hypothalamic-pituitary level.[1]

METHODOLOGY

The Access Total T4 assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel with anti-thyroxine antibody, thyroxine-alkaline phosphatase conjugate, and paramagnetic particles coated with goat anti-mouse capture antibody and a stripping agent to dissociate all T4 from binding proteins. Thyroxine in the sample competes with the thyroxine-alkaline phosphatase conjugate for binding sites on a limited amount of specific anti-thyroxine antibody. Resulting antigen: antibody complexes bind to the capture antibody on the solid phase.

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 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 thyroxine in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

SPECIMEN

SPECIMEN COLLECTION AND PREPARATION

1. Serum and plasma (heparin) are the recommended samples.
2. A study performed by Beckman Coulter, Inc. comparing heparin plasma to serum produced the following correlation statistics: $y = 0.9048x + 0.0236$, $r = 0.98$.
3. Observe the following recommendations for handling, processing, and storing blood samples:[3]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Physically separate serum or plasma from contact with cells as soon as possible.
   - 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 24 hours, or for shipment of samples, freeze at -20°C or colder.
   - Thaw samples only once.
4. 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.
5. 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.

REAGENTS

PRODUCT INFORMATION

Access Total T4 Reagent Pack

Cat. No. 33800: 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 14 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 pack is damaged (i.e., broken elastomer), discard the pack.
- All antisera are polyclonal unless otherwise indicated.

R1a:	Paramagnetic particles coated with goat anti-mouse IgG suspended in TRIS buffered saline, with surfactant, bovine serum albumin (BSA), 8-anilino-1-naphthalenesulfonic acid (ANS), < 0.1% sodium azide, and 0.1% ProClin* 300.
R1b:	Mouse monoclonal antibody to thyroxine diluted in TRIS buffered saline, with surfactant, protein (aves, murine, goat), < 0.1% sodium azide, and 0.1% ProClin 300.
R1c:	Thyroxine-alkaline phosphatase (bovine) conjugate diluted in TRIS buffered saline, with surfactant, protein (aves), < 0.1% sodium azide, and 0.1% ProClin 300.

*ProClin™ is a trademark of The Dow Chemical Company ("Dow") or an affiliated company of Dow.

WARNING 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.
- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

GXM Total T4 PMP (Compartment R1a)

WARNING

H317 May cause an allergic skin reaction.

	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) <0.05%
MxT4 Antibody (Compartment R1b)	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) <0.05%
Total T4 Conjugate (Compartment R1c)	WARNING	
		
	H317	May cause an allergic skin reaction.
	P261	Avoid breathing vapours.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) <0.05%

SDS	Safety Data Sheet is available at techdocs.beckmancoulter.com

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access Total T4 Calibrators
   Provided at zero and approximately 2.0, 4.0, 8.0, 16.0, and 30.0 µg/dL (26, 51, 103, 206, and 386 nmol/L).
   Cat. No. 33805
2. Quality Control (QC) materials: commercial control material
3. Access Substrate
   Cat. No. 81906
4. Access Wash Buffer II, Cat. No. A16792
   UniCel DxI Wash Buffer II, Cat. No. A16793

EQUIPMENT AND MATERIALS

R1	Access Total T4 Reagent Packs

CALIBRATION

CALIBRATION INFORMATION

An active calibration curve is required for all tests. For the Access Total T4 assay, calibration is required every 21 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.[4] 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.

TESTING PROCEDURE(S)

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 µg/dL. To change sample reporting units to the International System of Units (SI units), nmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply µg/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.

RESULTS INTERPRETATION

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.

REPORTING RESULTS

EXPECTED RESULTS

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.
2. Concentrations of total T4 were measured in serum samples from 533 presumably healthy subjects. The median value was 8.36 µg/dL (107.59 nmol/L) with a 95% non-parametric range of 6.09-12.23 µg/dL (78.38-157.40 nmol/L).

PROCEDURAL NOTES

LIMITATIONS

1. Samples can be accurately measured within the analytic range, between the lower limit of detection and the highest calibrator value (approximately 0.50-30.0 µg/dL [6.4-386 nmol/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., < 0.50 µg/dL [< 6.4 nmol/L]).
   - If a sample contains more than the stated value of the highest Access Total T4 Calibrator (S5), report the result as greater than that value (i.e., > 30.0 µg/dL [> 386 nmol/L]).
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.[5,6]

   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. The Access Total 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. Thyroid status should not be assessed on the basis of results from a single thyroxine test. Complete thyroid status evaluation should include additional thyroid function tests, evaluation of thyroid autoantibodies, and physician clinical evaluation.
5. In pregnancy, the Access Total T4 results may be incorrect, i.e., falsely-low. This assay should not be used as the only marker for thyroid disease evaluation during pregnancy. To ensure maximum diagnostic accuracy, thyroid status in pregnant women should be determined using thyroid function tests such as TSH, Free T4, Free Thyroxine Index (FTI) and clinical evaluation by the physician.[7]

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

METHODS COMPARISON

A comparison of serum thyroxine values using the Access Total T4 assay on the Access Immunoassay System and a commercially available enzyme immunoassay kit gave the following statistical data:

n	Range of Observations (µg/dL)	Intercept (µg/dL)	Slope	Correlation Coefficient (r)
164	1.44-22.45	0.65	0.95	0.97

A comparison of 151 values obtained by assaying paired serum (x) and plasma (y) samples using the Access Total T4 assay on the Access Immunoassay System gave the following statistical data:

n	Range of Observations (µg/dL)	Intercept (µg/dL)	Slope	Correlation Coefficient (r)
151	0.47-19.46	0.02	0.90	0.98

LINEARITY

Multiple dilutions of two samples containing various thyroxine levels with Access Total T4 Calibrator S0 (zero) resulted in the following data:

Sample 1	Expected Concentration (µg/dL)	Determined Concentration (µg/dL)	% of Expected
Neat	N/A	14.32	N/A
5/6	11.93	11.32	95
2/3	9.55	9.25	97
1/2	7.16	7.08	99
1/3	4.77	4.89	103
1/5	2.86	3.08	108
		Mean % Recovery	**100**

Sample 2	Expected Concentration (µg/dL)	Determined Concentration (µg/dL)	% of Expected
Neat	N/A	27.25	N/A
5/6	22.71	20.42	90
2/3	18.17	16.90	93

Sample 2	Expected Concentration (µg/dL)	Determined Concentration (µg/dL)	% of Expected
1/2	13.63	13.74	101
1/3	9.08	8.57	94
1/5	5.45	5.09	93
		Mean % Recovery	**94**

SPIKING RECOVERY

Addition of four different levels of thyroxine to two patient samples with low thyroxine resulted in the following data:

Sample 1 (µg/dL spike)	Expected Concentration (µg/dL)	Determined Concentration (µg/dL)	Recovery (%)
0	N/A	4.93	N/A
4	8.93	8.83	99
8	12.93	13.84	107
12	16.93	17.51	103
16	20.93	20.83	100
		Mean % Recovery	**102**

Sample 2 (µg/dL spike)	Expected Concentration (µg/dL)	Determined Concentration (µg/dL)	Recovery (%)
0	N/A	5.31	N/A
4	9.31	9.98	107
8	13.31	14.77	111
12	17.31	18.30	106
16	21.31	21.45	101
		Mean % Recovery	**106**

IMPRECISION

This assay exhibits total imprecision of less than 10% across the assay range. One study, using commercially available human serum based control material generating 2 or 3 assays per day, 3 replicates per assay, over 7 days provides the following data, analyzed via analysis of variance (ANOVA).[8,9]

Sample	Grand Mean (n=60) (µg/dL)	Within Run (%CV)	Total Imprecision (%CV)
Low	3.05	5.08	6.63
Medium	6.47	4.39	5.55
High	12.55	3.16	4.01

ANALYTICAL SPECIFICITY / INTERFERENCES

Samples containing up to 10 mg/dL (171 µmol/L) bilirubin, lipemic samples containing the equivalent of 1,800 mg/dL (20.32 mmol/L) triglycerides, hemolyzed samples containing up to 500 mg/dL (5 g/L) hemoglobin do not affect the concentration of thyroxine assayed.

The following table describes the cross-reactivity of the assay with substances that are similar in structure to T4.

Substance	Analyte Added (µg/dL)	Cross-Reactivity (%)
l-Thyroxine	5	100.00
d-Thyroxine	10	72.50
l-Triiodothyronine	500	1.24
d-Triiodothyronine	500	0.81
Tetraiodothyroacetic Acid	25	5.16
l-Diiodothyronine	5,000	0.08
d-Tyrosine	5,000	< 0.01
l-Tyrosine	5,000	< 0.01
Reverse T3	100	19.95
Monoiodotyrosine	5,000	0.01
Diiodotyrosine	5,000	< 0.01
Phenytoin	5,000	< 0.01
Phenylbutazone	5,000	< 0.01
6-*N*-Propyl-2-Thiouracil	5,000	0.01

ANALYTICAL SENSITIVITY

The lowest detectable level of thyroxine distinguishable from zero (Access Total T4 Calibrator S0) with 95% confidence is 0.50 µg/dL (6.4 nmol/L). 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 interpolated from the curve at the point that is two standard deviations from the mean measured zero calibrator signal.

ADDITIONAL INFORMATION

SYMBOLS KEY

Glossary of Symbols is available at techdocs.beckmancoulter.com (document number C02724)

REFERENCES

1. Gornall, AG, Luxton, AW, Bhavnani, BR. Endocrine disorders in applied biochemistry of clinical disorders, 305-318. Edited by Gornall, AG Philadelphia, PA: J B Lippincott Co, 1986.

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

3. Approved Guideline - Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests, GP44-A4. 2010. Clinical and Laboratory Standards Institute.

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

5. Kricka, L. Interferences in immunoassays - still a threat. Clin Chem 2000; 46: 1037-1038.

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

7. ACOG Practice Bulletin. Clinical management guidelines for obstetrician-gynecologists. Number 37, August 2002. (Replaces Practice Bulletin Number 32, November 2001). Thyroid disease in pregnancy. Obstet Gynecol. 2002 Aug; 100(2): 387-96.

8. Tentative Guideline - User evaluation of precision performance of clinical chemistry devices, EP5-T. National Committee for Clinical Laboratory Standards, 4(8) 1984.

9. Krouwer J.S., Rabinowitz R., How to improve estimates of imprecision, Clinical Chemistry, 30: 290-292: 1984

EC REP Beckman Coulter Eurocenter S.A., 22, rue Juste-Olivier. Case Postale 1044, CH - 1260 Nyon 1, Switzerland
Tel: +41 (0)22 365 36 11

Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.