BECKMAN COULTER

ACCESS
Immunoassay Systems

Instructions For Use

Access Free T3
Triiodothyronine, Free

REF A13422

FOR PROFESSIONAL USE ONLY

Rx Only

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

The Access Free T3 assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of free triiodothyronine 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 (hTSH). hTSH, in turn, stimulates the synthesis, storage, secretion, and metabolism of thyroxine (T4) and triiodothyronine (T3).

T3 is the major biologically active thyroid hormone. Of the circulating T3, about 80% is formed from peripheral deiodination of thyroxine and 20% is secreted directly from the thyroid gland. The T4 and T3 hormones are transported in the circulation bound to thyroxine binding globulin (TBG), thyroxine binding pre-Albumin (TPBA) and albumin. About 0.2 to 0.4% of the circulatory total T3 is in equilibria as unbound or free, in contrast to about 0.03% of the total T4. In most individuals, the free fractions of these hormones correlate with the functional thyroid state.[1,2]

Free T4 and 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]

With normal levels of thyroid binding proteins, free T3 levels correlate with total T3. Measuring free T3 is useful when altered levels of total T3 occur due to changes in thyroid hormone binding proteins, especially in cases with altered TBG or low albumin concentrations. Free T3 is elevated alone (T3 toxicosis) in about 5% of hyperthyroids.

Non-thyrometabolic disorders may cause abnormal free T3 levels. Determination of thyroid status in patients with non-thyroidal illness (NTI) should be interpreted with caution.[2,3] For example, anticonvulsant drug therapy (particularly phenytoin) may result in decreased free T3 levels due to an increased hepatic metabolism, and secondarily to

displacement of hormone from binding sites.[2,4,5] Anti-inflammatory drugs such as salicylate and phenylbutazone also compete for hormone binding sites, but their effect on free T3 levels has not been clearly defined.[2,6] Patients on heparin therapy may have elevated free T3 levels due to release of non esterified fatty acids (NEFA), which can alter the relationship between free and bound hormones.[5]

METHODOLOGY

The Access Free T3 assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel with an anti-T3 monoclonal antibody conjugated to alkaline phosphatase. During the incubation, free T3 in the sample reacts with the anti-T3 antibody. Particles coated with streptavidin and biotinylated T3 analog are then added to the mixture. Unoccupied binding sites on the anti-T3 antibody are bridged to the particle through the T3 analog.

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 free T3 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. Observe the following recommendations for handling, processing, and storing blood samples:[7]
   - 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 48 hours, or for shipment of samples, freeze at -20°C or colder.
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.
5. Thaw samples no more than three times.

REAGENTS

PRODUCT INFORMATION

Access Free T3 Reagent Pack

Cat. No. A13422: 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 pack 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, < 0.1% NaN_3 and 0.1% ProClin** 300.
R1b:	MES buffer and 0.1% ProClin 300.
R1c:	Biotinylated T3 analog in a TRIS buffer with protein (aves), surfactant, < 0.1% NaN_3 and 0.1% ProClin 300.
R1d:	TRIS buffer containing animal protein (goat, bovine, aves), surfactant, < 0.1% NaN_3, and 0.5% ProClin 300.
R1e:	Monoclonal antibody-alkaline phophatase conjugate in an ACES buffer with protein (aves), surfactant, < 0.1% NaN_3 and 0.1% ProClin 300.

*Dynabeads is a registered trademark of Dynal A.S., Oslo, Norway.

**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

FT3 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%
FT3 MES Buffer (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%
FT3 biotinylated 3,5-diiodothyronine (Compartment R1c)	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%
FT3 Blocking Reagent (Compartment R1d)	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%
Conjugate (Compartment R1e)	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%

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

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access Free T3 Calibrators
   Provided at zero and approximately 1, 2, 5, 10 and 30 pg/mL (1.5, 3.1, 7.7, 15 and 46 pmol/L).
   Cat. No. A13430
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 Free T3 Reagent Packs

CALIBRATION

CALIBRATION INFORMATION

An active calibration curve is required for all tests. For the Access Free T3 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.[8] 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 fifty-five (55) 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 pg/mL. 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 pg/mL by multiplication factor 1.536 (T3 m.w. equals 651 daltons).

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 free T3 were measured in sera from 200 apparently healthy, ambulatory subjects. The median value was 3.2 pg/mL (4.9 pmol/L) with a 95% non-parametric range of 2.5-3.9 pg/mL (3.8-6.0 pmol/L).

PROCEDURAL NOTES

LIMITATIONS

1. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.88-30 pg/mL [1.4-46 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., < 0.88 pg/mL [< 1.4 pmol/L]).
   - If a sample contains more than the stated value of the highest Access Free T3 Calibrator (S5), report the result as greater than that value (i.e., > 30 pg/mL [> 46 pmol/L]). Samples can not be diluted for free T3 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 or human anti-triiodothyronine antibodies may be present in patient samples.[9,10]
   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. The Access Free T3 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. This assay is susceptible to interference from high levels of biotin. The recommended daily intake for biotin is 30 µg. High doses of biotin (up to 30 mg per day) may be taken as a dietary supplement aimed at improving hair loss, nail growth, or skin condition.[11]

   Some pharmacokinetic studies have shown that serum concentrations of biotin can reach up to 300 ng/mL when subjects taking supplements containing 20 mg biotin[12] or 1,160 ng/mL for subjects taking doses of biotin up to 300 mg.[11] These studies were performed in apparently healthy subjects and some patients may be taking supplements with biotin at levels > 20 mg per day.

   Clearance rates of biotin may differ between patients tested with this device (e.g., patients with renal impairment), which may lead to higher than expected, or prolonged, concentrations in serum. If medically practicable, patients receiving biotin supplements should discontinue use prior to sample draws to minimize the risk of interference.

   Specimens with biotin concentrations ≤ 10 ng/mL demonstrated non-significant bias (≤ 10%) in results. Biotin concentrations > 10 ng/mL can lead to significant (> 10%) positive bias in Free T3 results.

   If unexpected results are obtained, and biotin interference is suspected, the Beckman Coulter TSH assay may provide a better assessment of thyroid function as it is not susceptible to biotin interference.
5. Serum and plasma values should not be used interchangeably. When monitoring a patient, it is suggested to rebaseline when changing sample types.

PERFORMANCE CHARACTERISTICS

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METHODS COMPARISON

A comparison of 397 values using the Access Free T3 assay on the Access Immunoassay system and a commercially available immunoassay kit gave the following statistical data using Deming calculations:[13]

n	Range of Observations (pg/mL)	Intercept (pg/mL)	Slope	Correlation Coefficient (r)
397	0.8-17.5	0.0828	0.9464	0.9874

IMPRECISION

This assay exhibits total imprecision ≤ 12% at concentrations greater than 2.0 pg/mL.

One study, using commercially available human serum based control material generating a total of 20 assays, 2 replicates per day, over 20 days provided the following data, analyzed via analysis of variance (ANOVA).[14,15]

Human Serum Control	Grand Mean (n=40) (pg/mL)	Within Run (%CV)	Between Run (%CV)	Total Imprecision (%CV)
Level 1	1.4	6.6	8.0	10.4
Level 2	2.6	2.6	5.1	5.7
Level 3	9.6	5.1	1.3	5.3

ANALYTICAL SPECIFICITY / INTERFERENCES

Samples containing up to 20 mg/dL (342 µmol/L) bilirubin, 10 mg/dL (119 µmol/L) conjugated bilirubin, lipemic samples containing the equivalent of 3,000 mg/dL (33.87 mmol/L) triolein, or up to 500 mg/dL cholesterol and samples containing up to 500 mg/dL (5.0 g/L) of hemoglobin do not effect the concentration of free T3 assayed.

The following substances were added to serum samples. When tested in the Access Free T3 assay, the observed mean percent changes were as follows:

Substance	Analyte Added (mg/dL)	% Change
Aspirin	75	1.3
Sodium Salycilate	75	13.9
Ibuprophen	750	17.2
Acetaminophen	200	-5.0
Phenylbutazone	7.5	3.9
Thiouracil	5	2.1
Phenytoin	10	-0.1
Furosemide	2	15.9
Carbamazepine	12	12.7
Methimazol	0.4	-0.1
Oleic Acid	283	11.4
Linoleic Acid	280	0

The following table describes the cross-reactivity of the assay with substances that are similar in structure to T3. Following CLSI C45A[16] recommendations the cross-reactants were tested in the absence of serum proteins (i.e. in HEPES pH 7.7 buffer containing 0.1% gelatin). The cross reactivities were calculated as the ratio between the concentration of T3 required to displace 50% of the conjugate (IC50) and the concentration of the cross-reactant required to give the same displacement, calculated on a molarity % basis.

Substance	Cross-reactivity (%)
Reverse T3	< 0.5
Tetraiodothyroacetic acid	< 0.5
D-thyroxine	< 0.5
L-thyroxine	< 0.1
3, 5 diiodothyronine	3.3
Diiodo-L-tyrosine	< 0.01
Monoiodotyrosine	< 0.01
3-3', 5-Triiodothyroacetic acid (TRIAC)	13.0

Biotin

The following concentrations of biotin were spiked into normal serum samples at high and low Free T3 concentrations and found to give the following percent change in result response.

		Biotin (ng/mL)					
Analyte Range	Analyte Concentration	10	25	100	300	600	1,200
Low	2.62 - 2.69 pg/mL	9%	19%	121%	184%	258%	395%
High	2.9 - 3.0 pg/mL	4%	12%	106%	194%	288%	417%

ANALYTICAL SENSITIVITY

The lowest detectable level of free T3 distinguishable from zero (Access Free T3 Calibrator S0) with 95% confidence is 0.88 pg/mL (1.4 pmol/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)

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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.