

IVD For In Vitro Diagnostic Use

Rx Only

REF 100050 (39 x 24 mL Kits)

Intended Use

The CEDIA™ T4 assay is an in-vitro diagnostic medical device intended for the quantitative determination of total thyroxin (T4) in serum and plasma.

Summary and Explanation of The Test

Thyroxin (T4) and triiodothyronine (T3) are thyroid hormones that are essential in the regulation of various metabolic functions.^{1,2} They are synthesized in the thyroid gland from iodine and the amino acid tyrosine, and their synthesis and release into the circulation are regulated through a complex negative feedback system involving the hypothalamus, pituitary and thyroid glands. The hypothalamus releases thyrotropin releasing hormone (TRH) which stimulates the pituitary to release thyroid stimulating hormone (TSH). TSH in turn causes the thyroid to secrete T3 and T4. Increased levels of thyroid hormones then act on the anterior pituitary to decrease TSH release, creating a self-regulating feedback system.^{3,4}

Approximately 70-100 µg of T4 are secreted into the circulation daily. Once in circulation, T4 is found predominantly bound to the plasma protein thyroxin-binding globulin, though to a lesser extent it also binds to thyroxin-binding prealbumin and in some cases, albumin. Less than 1% of thyroxin is found free or unbound in plasma.^{1,2,5}

Increases in total thyroxin are seen in hyperthyroidism, a condition caused by an excess of circulating hormone, and decreased levels are seen in hypothyroidism, a condition caused by insufficient amounts of circulating hormone. Quantitation of total thyroxin levels, along with clinical history and other thyroid function tests such as T Uptake, is a valuable tool in the evaluation of thyroid function.^{2,4,6}

The CEDIA T4 assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.⁷

The assay is based on the bacterial enzyme β -galactosidase, which has been genetically engineered into two inactive fragments i.e., enzyme acceptor (EA) and enzyme donor (ED). These fragments spontaneously reassociate to form fully active enzyme that, in the assay format, cleaves a substrate, generating a color change that can be measured spectrophotometrically. In the assay, analyte in the sample competes with analyte conjugated to one inactive fragment of β -galactosidase for antibody binding site. If analyte is present in the sample, it binds to antibody, leaving the inactive enzyme fragments free to form active enzyme. If analyte is not present in the sample, antibody binds to analyte conjugated on the inactive fragment, inhibiting the reassociation of inactive β -galactosidase fragments, and no active enzyme is formed. The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of analyte present in the sample.

Reagents

- 1 **EA Reconstitution Buffer:** Contains 1.25 mg/L monoclonal anti-T4 antibody, 63 mg/L ANS, 3-(N-morpholino) propanesulfonic acid, buffer salt, stabilizers, preservative, (39 mL).
- 1a **EA Reagent:** Contains 0.19 g/L enzyme acceptor, buffer salt, detergent and preservative.
- 2 **ED Reconstitution Buffer:** Contains 3-(N-morpholino) propanesulfonic acid, buffer salt and preservative, (24 mL).
- 2a **ED Reagent:** Contains 0.44 mg/L enzyme donor conjugated to thyroxin, 3.27 g/L o-nitrophenyl- β -D-galactopyranoside, stabilizers and preservative.
- 3 **Low Calibrator:** Contains thyroxin, bovine serum albumin, buffer salt and preservative.
- 4 **High Calibrator:** Contains thyroxin, bovine serum albumin, buffer salt and preservative.

⚠ Precautions and Warnings

DANGER:

CEDIA T4 Assay Powder Reagents contain BSA & Sodium azide.
CEDIA T4 Assay Liquid Reagents contain Sodium azide.
CEDIA T4 Assay Calibrators contain BSA & Sodium azide.

H317 - May cause allergic skin reaction.

H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.

EUH032 - Contact with acids liberates very toxic gas.

Avoid breathing dust/mist/vapors/spray. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/ eye protection/face protection. In case of inadequate ventilation wear respiratory protection. If on skin: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. If skin irritation or rash occurs: Get medical advice/attention. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. Wash contaminated clothing before reuse. Dispose of contents/ container to location in accordance with local/regional/national/international regulations.

The reagents contain sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes or if ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10% sodium hydroxide.

Reagent Preparation and Storage

Remove the kit from refrigerated storage immediately prior to preparation of the working solutions.

Prepare the solutions in the following order to minimize possible contamination.

R2 Enzyme donor solution: Connect Bottle 2a (ED Reagent) to Bottle 2 (ED Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 2a is transferred into Bottle 2. Avoid the formation of foam. Detach Bottle 2a and adapter from Bottle 2 and discard. Cap Bottle 2 and let stand approximately 5 minutes at 15-25°C. Mix again. Record the reconstitution date on the bottle label. Place the bottle directly into the reagent compartment of the analyzer or into refrigerated storage and let stand 30 minutes before use.

R1 Enzyme acceptor solution: Connect Bottle 1a (EA Reagent) to Bottle 1 (EA Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 1a is transferred into Bottle 1. Avoid the formation of foam. Detach Bottle 1a and adapter from Bottle 1 and discard. Cap Bottle 1 and let stand approximately 5 minutes at 15-25°C. Mix again. Record the reconstitution date on the bottle label. Place the bottle directly into the reagent compartment of the analyzer or into refrigerated storage and let stand 30 minutes before use.

Calibrators: Reconstitute Bottle 3 and Bottle 4 with the appropriate amount of distilled or deionized water (4.0 mL each). Swirl gently at 15-25°C for 15 minutes. Avoid the formation of foam. Ensure complete dissolution before use. Record the reconstitution date on the bottle labels.

NOTE 1: The components supplied in this kit are intended for use as an integral unit. Do not mix components from different lots.

NOTE 2: Avoid cross-contamination of reagents by matching reagent caps to the proper reagent bottle. The R2 Working Solution (Enzyme Donor) should be colorless to pale yellow in color. A yellow or yellow-orange color indicates that the reagent has been contaminated and must be discarded.

NOTE 3: The R1 and R2 Working Solutions must be at the reagent compartment temperature of the analyzer before performing the assay.

NOTE 4: To ensure reconstituted EA reagent stability, protect from prolonged continuous exposure to bright light.

Store reagents at 2-8°C. **DO NOT FREEZE.** For stability of the unopened components, refer to the box or bottle labels for the expiration date.

R1 Solution: 45 days refrigerated on analyzer or at 2-8°C.

R2 Solution: 45 days refrigerated on analyzer or at 2-8°C.

Reconstituted Calibrators: 45 days at 2-8°C.

Specimen Collection and Handling

Serum or plasma (Na or Li heparin; Na EDTA) samples are suitable for use in the assay. Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the sample from the time it is collected until the time it is assayed. Centrifuge specimens containing particulate matter. Cap samples, store at 2-8°C and assay within 10 days after collection. If the assay cannot be performed within 10 days, or if the sample is to be shipped, cap the sample and keep it frozen. Samples can be stored at -20°C for up to 6 months. **Handle all patient samples as if they were potentially infectious.**

Assay Procedure

Chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzymatic rates and timing the reaction accurately can be used to perform this assay. Application sheets with specific instrument parameters are available from Microgenics, a part of Thermo Fisher Scientific.

NOTE: If the bar code is not read by the analyzer, the numerical sequence on the bar code label can be entered manually via the keyboard.

Quality Control and Calibration⁸

Recalibration is recommended

- as blank calibration every 12 hours
- as 2 point calibration every 5 days if the reagent bottles are on analyzer for more than 5 days
- as 2 point calibration after reagent bottle change
- as 2 point calibration after reagent lot change
- as 2 point calibration as required following quality control procedures

For quality control use Precitrol-N and Precitrol-A, Precinorm TDM or other suitable control material.

Good laboratory practice suggests that controls be run each day patient samples are assayed and each time a calibration is performed. Monitor the control values for any trends or shifts. If any trends or shifts are detected, or if the control values do not recover within the specified range, review all operating parameters. Contact Technical Support for further assistance. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Results and Expected Values

The CEDIA T4 Assay quantitates patient samples having T4 concentrations ranging from 0.5 - 20 µg/dL. Specimens yielding results greater than 20 µg/dL can be diluted 1 + 1 with Low Calibrator and reassayed. Multiply the results by 2 and subtract the concentration of the Low Calibrator to obtain the sample T4 concentration. A T4 range of 4.5 - 12 g/dL⁸ was obtained from 301 blood donors tested using the CEDIA T4 Assay. Other normal T4 ranges such as 4.5 - 13.2 µg/dL⁹ and 5 - 12 µg/dL⁴ have been reported. Each laboratory should establish its own T4 concentration ranges based on its sample population. The following conversion factor can be used to convert µg/dL to nmol/L:

$$\mu\text{g/dL} \times 12.9 = \text{nmol/L}$$

Elevated levels of T4 can result from increased hormone synthesis, increased hormone release from thyroid cells, and increased binding capacity of plasma proteins, in particular thyroxin-binding globulin (TBG). Examples of hyperthyroidism include Graves' disease, toxic multinodular goiter, and toxic thyroid adenoma. T4 levels will also be increased in pregnancy and in women taking oral contraceptives containing estrogen. Decreased levels of T4 occur in primary, secondary, and tertiary hypothyroidism. These include myxedema, cretinism, and nephrosis.^{1,2,9}

The free thyroxin index (FTI) is a means of normalizing the effects of thyroxin-binding proteins on total T4 levels. The FTI yields a value which is related to the biologically active free T4 concentration.^{5,10}

Total thyroxin tests should be used in conjunction with a T Uptake test to determine the free thyroxin index. It is recommended that the Microgenics T4 Reagents be used with the Microgenics T Uptake Reagents for FTI determinations.

Limitations

1. The incidence of patients with antibodies to *E. coli* β -galactosidase is extremely low. However, some samples containing such antibodies can result in artificially high T4 results that do not fit the clinical profile.
2. As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample, which could cause falsely elevated results.

Specific Performance Characteristics

The following data were determined using a Hitachi system.¹¹ Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using controls in an internal protocol. Within run imprecision was determined by assaying 21 replicates in a single run. Between day imprecision was determined by single point quantitation in 21 separate runs. The following results were obtained.

Sample	Within-run			Between day		
	\bar{x}	SD	CV%	\bar{x}	SD	CV%
	µg/dL	µg/dL		µg/dL	µg/dL	
Control 1	3.4	0.18	5.1	3.7	0.34	9.2
Control 2	8.6	0.30	3.4	8.9	0.35	3.9
Control 3	16.3	0.28	1.7	17.1	0.45	2.6

Method Comparison

A comparison using the Microgenics CEDIA T4 assay (y) with a commercial fluorescence polarization immunoassay (x) gave the following correlation (µg/dL):

Deming's

$$y = -0.02 + 1.05x$$

$$r = 0.990$$

$$Sy.x = 0.44$$

Linear regression

$$y = 0.06 + 1.04x$$

$$r = 0.990$$

$$Sy.x = 0.62$$

Number of samples measured: 114

The sample concentrations were between 1.2 and 17.7 µg/dL.

Linearity

A high sample was diluted with the CEDIA T4 Low Calibrator. The percent recovery was then determined by dividing the assayed value by the expected value.

% High Sample	Expected Value (µg/dL)	Assayed Value (µg/dL)	% Recovery
100.0	-	19.70	-
87.5	17.26	16.98	98
75.0	14.88	15.07	101
62.5	12.48	12.21	98
50.0	10.07	10.05	100
37.5	7.67	7.49	98
25.0	5.26	5.22	99
12.5	2.86	3.06	107
0.0	-	0.45	-

Recovery

Thyroxin in the form of high (spiked) patient sample was added to a low patient sample. The percent recovery was then determined by dividing the assayed value by the expected value.

% High Sample	Expected Value (µg/dL)	Assayed Value (µg/dL)	% Recovery
100.0	-	20.60	-
87.5	18.43	18.54	101
75.0	16.27	16.68	103
62.5	14.10	14.12	100
50.0	11.93	11.91	100
37.5	9.77	9.85	101
25.0	7.64	7.64	101
12.5	5.43	5.43	100
0.0	-	3.26	-

Specificity

The CEDIA T4 assay is highly specific, having very low cross-reactivity to similar amino acids and drugs. Cross-reactivity was 90% for D-Thyroxin and was clinically insignificant for the following compounds:

$$3,3',5\text{-Triiodothyroacetic acid}$$

$$3,3',5\text{-Triiodo-L-thyronine (T3)}$$

$$3,3',5\text{-Triiodo-D-thyronine (D-T3)}$$

There was negligible cross-reactivity (< 0.1%) with the following compounds:

$$\text{Acetylsalicylic acid}$$

$$\text{Methimazole}$$

$$\text{Phenylbutazone}$$

$$\text{Salicylic acid}$$

$$5,5'\text{-Diphenylhydantoin}$$

$$\text{DL-Tyrosine}$$

$$6\text{-n-Propyl-2-thiouracil}$$

$$3,5\text{-Diiodo-L-thyronine}$$

$$3\text{-Iodo-L-tyrosine}$$

The following substances showed no interference with the CEDIA T4 Assay:

Substance	Concentration	Substance	Concentration
Bilirubin	≤ 60 mg/dL	Total protein	≤ 12 g/dL
Hemoglobin	≤ 0.55 g/dL	Triglyceride	≤ 2.0 g/dL
Rheumatoid factor	≤ 96 IU/mL		

Sensitivity

The assay limit of detection is 0.5 µg/dL.

References

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Glossary:

<http://www.thermofisher.com/symbols-glossary>



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