# CEDIA® T Uptake

Thermo SCIENTIFIC

**IVD** **For In Vitro Diagnostic Use**

**Rx Only**

**REF** 100049

## Intended use
The CEDIA® T Uptake assay is for the quantitative determination of unoccupied binding sites of thyroxine-binding proteins in serum and plasma on automated clinical chemistry analyzers. T Uptake measurements are used in the diagnosis and treatment of thyroid disorders.

## Summary
Thyroxine (T4) and triiodothyronine (T3) are hormones derived from the thyroid gland which act to regulate a wide variety of metabolic functions.[1,2] Over 99% of circulating T4 is bound to three plasma proteins: thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA) and albumin. Approximately 60% of thyroxine is bound to TBG, 30% to TBPA and about 10% to albumin. Less than 1% of thyroxine is unbound or free and thus is available for biological activity.[3,4] Bound T4 is in equilibrium with free T4 and unoccupied thyroid hormone binding sites; a similar equilibrium exists with T3. Approximately two thirds of TBG binding sites are unoccupied and available for binding free hormone. Changes in the concentration of thyroid-binding proteins affect free hormone levels, triggering the depression or stimulation of hormone production until a new equilibrium is reached. Therefore, the clinical usefulness of total thyroid hormone measurement is compromised if there are marked changes in the binding capacity or concentration of thyroxine-binding proteins.[4]

The concentration of unoccupied thyroid-binding sites is often determined by T Uptake assays. In these tests, labeled T4 or T3 is added to a specimen and the percent of unbound labeled hormone is measured. This value can then be used to adjust the total T4 concentration for variations in the thyroid-binding capacity. For a given sample, multiplying the T4 concentration by the T Uptake value (often normalized to the mean value of a population of healthy individuals) produces a Free Thyroxine Index (FTI), which is closely correlated to the free T4 concentration as measured by more direct methods.[5]

T Uptake tests have classically been performed using radioisotopes to determine the saturation of thyroxine-binding proteins. These are indirect tests which measure radiolabeled tracer bound to a secondary binder, such as erythrocyte membranes, resin, silica, charcoal or antibody. Additionally, these tests involve separation steps which often require centrifugation.[6,7] This homogeneous enzyme immunoassay is rapid, easily automated and requires no separation steps or secondary binders.

## Reagents
- **1** **EA Reconstitution Buffer,** 2 x 588 mL
- **1a** **EA Reagent,** 2 for 588 mL
- **2** **ED Reconstitution Buffer,** 2 x 191 mL
- **2a** **ED Reagent,** 2 for 191 mL
- **3** **Hypothyroid Calibrator,** 2 for 20 mL
- **4** **Hyperthyroid Calibrator,** 2 for 20 mL

## Test principle[8]
The CEDIA T Uptake assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.

The assay is based on the bacterial enzyme ß-galactosidase, which has been genetically engineered into two inactive fragments. 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, enzyme donor-thyroxine conjugate binds directly to the unoccupied thyroxine-binding sites in the sample, preventing the spontaneous reassociation of the enzyme fragments to form active enzyme. Thus, thyroxine-binding proteins regulate the amount of ß-galactosidase formed. The unoccupied thyroxine-binding protein sites in the samples are inversely proportional to the amount of ß-galactosidase formed from the reassembly of the remaining enzyme donor and enzyme acceptor as monitored by the hydrolysis of the substrate o-nitrophenyl-ß-D-galactopyranoside (ONPG).

## Working solution concentration
See below for working solution concentrations for Hitachi analyzers. For other analyzers, refer to the instrument-specific application sheet.

***R1 EA Working Solution***
enzyme acceptor (microbial): 0.111 g/L; phosphate buffer; buffer salts; stabilizers; preservative; detergent.
***R2 ED Working Solution***
enzyme donor (microbial)-thyroxine conjugate: 0.44 mg/L; o-nitrophenyl-ß-D-galactopyranoside: 3.27 g/L; phosphate buffer; buffer salts; stabilizers; detergent; preservative
***3 Hypothyroid Calibrator***
thyroxine-binding globulin: 65 mg/L; human serum matrix; stabilizer; preservative.
See bottle label for lot specific value.
***4 Hyperthyroid Calibrator***
thyroxine: 0.25 µg/mL; human serum matrix; stabilizer; preservative.
See bottle label for lot specific value.

## ⚠ Precautions and warnings
For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents.

**DANGER:** CEDIA T Uptake Reagents contains:

**Reagents 1 and 2** - EA and ED Reconstitution Buffers contain ≤0.15% Sodium azide.
EUH032 - Contact with acids liberates very toxic gas.

**Reagent 1a** - EA Reagent contains ≤1% Sodium azide.
H412 - Harmful to aquatic life with long lasting effects.
EUH032 - Contact with acids liberates very toxic gas.

Avoid release to the environment. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

**Reagent 2a** - ED Reagent contains ≤17% Sodium phosphate, monobasic; ≤15% Bovine serum albumin (BSA); and ≤0.5% Sodium azide.
H315 - Causes skin irritation.
H317 - May cause allergic skin reaction.
H319 - Causes serious eye irritation.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H412 - Harmful to aquatic life with long lasting effects.
EUH032 - Contact with acids liberates very toxic gas.

Avoid breathing dust. Avoid release to the environment. 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 IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If skin irritation or rash occurs: Get medical advice/attention. If eye irritation persists: Get medical advice/attention. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. Take off contaminated clothing and wash before reuse. Wash contaminated clothing before reuse. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

**Reagents 3 and 4** - Hypothyroid and Hyperthyroid Calibrators contain ≤1.5% Sodium azide.
H302 - Harmful if swallowed.
H412 - Harmful to aquatic life with long lasting effects.
EUH032 - Contact with acids liberates very toxic gas.

Wash hands thoroughly after handling. Do not eat, drink or smoke when using this product. Avoid release to the environment. IF SWALLOWED: Call a Poison Center or doctor/physician if you feel unwell. Rinse mouth. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

Materials of human origin have been tested for HIV 1 and 2, hepatitis B and hepatitis C infections. The findings were negative. However, as no testing method can rule out the risk of potential infection with absolute certainty, the materials must be handled just as carefully as patient specimens. In the event of exposure, the directives of the responsible health authorities should be followed.[9,10]

## Reagent handling
Refer to the instrument-specific application sheet.

Prepare the working solutions using cold reagents and buffers. Remove the kit from refrigerated storage (2-8°C) immediately prior to preparation of the working solutions.

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

***R2:*** 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 (2-8°C) and let stand 5 minutes before use.
***R1:*** 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 (2-8°C) and let stand 5 minutes before use.

***Calibrators:*** Reconstitute Bottle 3 (Hypothyroid Calibrator) and Bottle 4 (Hyperthyroid Calibrator) with the appropriate amount of distilled or deionized water (4.0 ml for Catalog No. 100047, 20.0 ). Swirl gently at 15-25°C for 45 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 deep 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.

## Storage and stability
Unopened kit components: up to the expiration date at 2-8°C. **Do not freeze.**

**R1 and R2:** 30 days opened and refrigerated on the analyzer or at 2-8°C. **Do not freeze.**
**Reconstituted Bottle 3 (Hypothyroid Calibrator) and Bottle 4 (Hyperthyroid Calibrator):** 30 days at 2-8°C. **Do not freeze.**

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

## Specimen collection and preparation
Collect serum using standard sampling tubes.
Sodium or lithium heparin or sodium EDTA plasma may be used.

Stability: 10 days capped at 2-8°C
4 weeks capped at -20°C

Avoid repeated freezing and thawing. Do not induce foaming of specimens. Centrifuge samples containing precipitate before performing the assay.

## Testing procedure
***Materials provided***
- Working solutions as described above
- Hypothyroid and Hyperthyroid Calibrators as described above

***Additional materials required***
- Controls as indicated below
- Distilled or deionized water and volumetric pipet for calibrator reconstitution

## Assay
Refer to the instrument-specific application sheet for analyzer specific assay instructions. The performance of applications not validated by Microgenics is not warranted and must be defined by the user.

## Calibration
**S1:** CEDIA T Uptake Hypothyroid Calibrator
**S2:** CEDIA T Uptake Hyperthyroid Calibrator

***Calibration frequency***
See below for calibration frequency recommendations for Hitachi analyzers. For other analyzers, refer to the instrument-specific application sheet.

2 point calibration is recommended
- every 4 days if the reagent bottles are on the analyzer for more than 4 days
- after reagent bottle
- after reagent lot change
- as required following quality control procedures

Calibration verification: Not necessary.

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

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements.

Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

## Calculation
Hitachi systems automatically calculate the T Uptake concentration of each sample.

## Limitations - interference[11]
**Criterion:** recovery +10% of initial value
Icterus: No significant interference up to an I index of 60 (approximate bilirubin concentration: 60 mg/dL).

**Hemolysis:** No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL).

Interference studies for lipemia were performed by conducting a method comparison with the previous T Uptake assay, using native specimens containing up to 1000 mg/dl triglycerides. No significant bias was observed.

This assay was validated on Hitachi analyzers utilizing an integral cell wash. If your analyzer does not have an integral cell wash, contact your local Thermo Fisher Scientific representative for an alternative procedure.

To alleviate probe carryover into the CEDIA Cortisol assay, refer to the instrument-specific instructions for additional information. (US users refer to application sheet.)

The incidence of patients with antibodies to E. coli ß-galactosidase is extremely low. However, some samples containing such antibodies can result in artificially high results that do not fit the clinical profile. If this occurs, contact Customer Technical Support.

## Measuring/reportable range
15 - 50%
**NOTE:** Specimens above this range should not be diluted.

## Expected values
24.3 - 39.0%[11]
This range was determined using a population of 400 blood donors. The samples were run on a Hitachi analyzer using CEDIA T Uptake reagents.

T Uptake levels, like total T4 levels, are generally increased in hyperthyroidism and decreased in hypothyroidism. However, in individuals who are euthyroid in terms of free T4, alterations in thyroxine-binding proteins result in opposite changes in % T Uptake and total T4 levels. For instance, in euthyroid conditions such as pregnancy or estrogen therapy, binding protein and total T4 levels are increased and T Uptake levels are decreased.[1]

Alterations in Plasma Concentration of Thyroxine-Binding Proteins[12]
**Decreased levels in:**
Treatment with anabolic steroids, androgens, diphenylhydantoin (phenytoin)
Major illness or surgical stress
Nephrotic syndrome
Active acromegaly
Genetic (inherited) deficiency
**Increased levels in:**
Treatment with estrogens, perphenazine
Pregnant or newborn state
Acute intermittent porphyria
Infectious hepatitis
Genetic (inherited) increase in synthesis

The free thyroxine index (FTI) is a means of normalizing the effects of thyroxine-binding proteins on total T4 levels. The FTI yields a value which is related to the biologically active free T4 concentration.[13-15]

The FTI may be calculated as follows:[16]
FTI = T4 • T Uptake Ratio

where T Uptake Ratio is defined as:

$$\text{T Uptake Ratio} = \frac{\text{T Uptake Value}}{\text{Mean Normal T Uptake Value}}$$

Mean Normal T Uptake Value in the above equation refers to the mean value of the normal range study. A mean normal T Uptake value of 31.7% was used in these calculations. The normal range of FTI values as defined by the mean ± two standard deviations is 4.6 to 11.7.

The FTI may also be calculated as follows:[1]

$$\text{FTI} = \frac{\text{Total T4 x T Uptake}}{100}$$

Using this calculation, the normal range of FTI values is 1.5 - 3.8.

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

Each laboratory should establish transferability of the expected values to its own patient population and if necessary determine its own reference range.

For diagnostic purposes, T Uptake results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

## Specific performance data[11]
The data determined using a Hitachi system are given below. Results obtained in individual laboratories may differ.

## Imprecision

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 35 separate runs. The following results were obtained.

| Sample | Within-run | | | Between day | | |
|---|---|---|---|---|---|---|
| | Mean | SD | %CV | Mean | SD | %CV |
| | % | % | | % | % | |
| **Control 1** | 29.8 | 0.25 | 0.9 | 30.7 | 0.48 | 1.5 |
| **Control 2** | 39.5 | 0.21 | 0.5 | 40.2 | 0.52 | 1.3 |
| **Control 3** | 48.8 | 0.48 | 1.0 | 48.9 | 0.52 | 1.1 |

## Method comparison

A comparison using this CEDIA T Uptake assay (y) with the previous CEDIA T Uptake assay (x) gave the following correlation (%):

| Deming's | Linear regression |
|---|---|
| y = 0.77 + 0.97 x | y = 1.00 + 0.96 x |
| r = 0.992 | r = 0.992 |
| Sy.x = 0.58 | Sy.x = 0.81 |

Number of samples measured: 432
The sample concentrations were between 14.8 and 49.2%.

## References


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## Instrument settings

Refer to application sheet for additional operating information.

## Glossary:

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


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For insert updates go to:
www.thermoscientific.com/diagnostics

**Other countries:**
Please contact your Thermo Fisher Scientific representative.

For detailed information, consult the operator manuals for Hitachi systems, the respective application sheets and the package inserts for the control sera.




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