

**Total T4 (Thyroxine)****Order information**

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
20739006 322	Total T <sub>4</sub> (Thyroxine) (275 tests)	System-ID 07 3900 6 COBAS INTEGRA 400 plus COBAS INTEGRA 800
20762016 322	COBAS FP Total T <sub>4</sub> Calibrators A (1 × 3.5 mL), B-F (5 × 1.5 mL)	System-ID 07 6201 6 US # 46201
12149435 122	Precinorm U plus (10 × 3 mL)	System-ID 07 7999 7
12149435 160	Precinorm U plus (10 × 3 mL, for USA)	System-ID 07 7999 7
12149443 122	Precipath U plus (10 × 3 mL)	System-ID 07 8000 6
12149443 160	Precipath U plus (10 × 3 mL, for USA)	System-ID 07 8000 6
20720720 322	COBAS FP SDR II (1 × 200 mL)	System-ID 07 2072 0 US # 44268
20764337 322	COBAS INTEGRA Cleaner Cassette (150 tests)	System-ID 07 6433 7

**English****System information**

Test T4, test ID 0-900.

**Intended use**

In vitro test for the quantitative determination of total T<sub>4</sub> (free and protein bound) in human serum or plasma prepared with heparin or oxalate on COBAS INTEGRA systems.

**Summary**

Thyroxine (T<sub>4</sub>) is the most abundant of the iodinated hormones secreted by the thyroid gland.<sup>1,2</sup> Most of the serum T<sub>4</sub> is bound to proteins, primarily thyroxine binding globulin (TBG), which transport the hormone to its sites of action.<sup>3,4</sup> A small fraction of T<sub>4</sub> is free in the circulatory system and is thought to be the biologically active material that can enter cells as needed to maintain a balanced tissue-bound fraction of T<sub>4</sub>. T<sub>4</sub> secretion is stimulated by thyroid stimulating hormone (TSH), and hormone levels are regulated by a negative feedback system to the pituitary gland.<sup>5</sup> Abnormalities in T<sub>4</sub> secretion can have profound effects in energy production or fat or protein metabolism. Clinical symptoms such as seen in Graves disease are associated with hyperthyroidism, and hypothyroidism can be associated with goiters and cretinism.<sup>6,7,8,9,10</sup> Changes in TBG concentration can make thyroid assessment difficult. Such changes may occur, for example, with pregnancy, estrogen treatment, and usage of drugs such as phenytoin and salicylates which compete for T<sub>4</sub> binding sites on serum proteins. Under these conditions it is best to use the total T<sub>4</sub> assay in conjunction with other thyroid function tests. For example, a total T<sub>4</sub> run together with T-uptake will generate a free thyroxine index (FTI).<sup>11,12</sup>

**Test principle**

Fluorescence polarization.

COBAS INTEGRA Total T<sub>4</sub> (Thyroxine) measurements are made on COBAS INTEGRA systems using the principle of fluorescence polarization. When a fluorescent molecule, or fluorophore, is irradiated with light of the proper wavelength (the excitation wavelength) some of the light is absorbed. Within a few nanoseconds the absorbed light is emitted, although at a longer wavelength (the emission wavelength). Whether or not the emitted light is polarized depends on the freedom of the fluorophore to rotate in solution. A small molecule, such as fluorescein, can rotate rapidly before light emission occurs, resulting in depolarization of the emitted light. In contrast, a fluorescent macromolecule, such as a fluorescein-labeled protein, will rotate much more slowly. Thus, in the time frame between excitation and emission, the macromolecule will have rotated only very slightly and the emitted light will be polarized.<sup>13</sup>

Fluorescence polarization is a reproducible function of the hormone concentration, and is suitable for the quantitative determination of total T<sub>4</sub> concentrations in serum for the purpose of thyroid monitoring.

Surface active agents are used to ensure dissociation of the hormone from serum proteins and to prevent nonspecific binding of the tracer.

**Reagents - working solutions****Note**

Use only the products described in this method sheet. The use of other reagents, calibrators, or standards may produce erroneous results.

**R1** Antibody reagent: Anti-T<sub>4</sub> monoclonal antibody (mouse) in buffer, pH 7.5; stabilizer; preservative.

**SR** Tracer reagent: Fluorescein-labeled T<sub>4</sub> derivative in buffer, pH 8.5; stabilizer; preservative.

R1 is in position B and SR is in position C.

**Precautions and warnings**

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: For prescription use only.

**Reagent handling**

Ready for use

**Storage and stability**

Shelf life at 2-8 °C

See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C

12 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C

26 weeks

Do not use reagents after the expiration date on the **cobas c** pack label. Failure to obtain the proper range of results in the assay of control material may indicate reagent deterioration.

**Specimen collection and preparation**

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

**Serum:** Collect serum using standard sampling tubes. Serum is the specimen of choice.

**Plasma:** Li-heparin or oxalate plasma. Do not use EDTA or fluoride plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Specimens should not be repeatedly frozen and thawed. Thawed specimens should be inverted several times prior to testing.

Stability:

8 hours at 15-25 °C

up to 48 hours at 2-8 °C

longer periods at (-20) °C or below

Centrifuge samples containing precipitates before performing the assay.

**Materials provided**

See "Reagents – working solutions" section for reagents.

**Total T4 (Thyroxine)****Materials required (but not provided)**

COBAS FP SDR II, Cat. No. 20720720, System-ID 07 2072 0.

The SDR II is placed as special diluent in its predefined rack position. It is stable on-board for 7 days.

**Assay**

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

**Application for serum and plasma****COBAS INTEGRA 400 plus test definition**

Measuring mode		FP
Reaction mode		R1-SDR/S-SR
Wavelength	excitation	485 nm
	emission	515 nm
Reading cycle blank/test		29/45
Unit		µg/dL

**Pipetting parameters**

		Diluent (H <sub>2</sub> O)
R1	105 µL	10 µL
Sample	3.5 µL	5 µL
Special diluent (SDR)	49 µL	
SR	15 µL	10 µL
Total volume	197.5 µL	

**COBAS INTEGRA 800 test definition**

Measuring mode		FP
Reaction mode		R1-SDR/S-SR
Wavelength	excitation	485 nm
	emission	515 nm
Reading cycle blank/test		40/60
Unit		µg/dL

**Pipetting parameters**

		Diluent (H <sub>2</sub> O)
R1	105 µL	10 µL
Sample	3.5 µL	5 µL
Special diluent (SDR)	49 µL	
SR	15 µL	10 µL
Total volume	197.5 µL	

**Calibration**

Calibrator	6 COBAS FP Total T <sub>4</sub> Calibrators
Total T <sub>4</sub> conc.	0, 3, 6, 12, 18, 24 µg/dL (0, 39, 77, 155, 232, 310 nmol/L)
Calibration mode	Logit/log 4
Calibration replicate	Duplicate recommended
Deviation low/high	< 10 % at ≥ 3 µg/dL (≥ 39 nmol/L)
Calibration interval	Each lot and 20 weeks and as required following quality control procedures

A calibration curve must be prepared using the COBAS FP Total T<sub>4</sub> Calibrators. Calibrators must be placed from the highest concentration (F) first, to the lowest (A) last, on the CAL/QC rack.

This curve is retained in memory by the COBAS INTEGRA systems and recalled for later use.

**Quality control**

Quality control	Precinorm U plus Precipath U plus
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

**Calculation**

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factor: µg/dL × 12.9 = nmol/L

**Limitations - interference**

Specimens with assay values greater than the highest calibrator will be flagged by the system and must be repeated after appropriate dilution of the original sample with the zero calibrator. Specimens with high fluorescent backgrounds or those giving polarization values greater than the zero calibrator will also be flagged by the system.

Criterion: Recovery within ± 10 % of initial value.

At 10 µg/dL (129 nmol/L) T<sub>4</sub>:

Icterus:<sup>14</sup> No significant interference up to an I index of 15 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 257 µmol/L or 15 mg/dL).

Hemolysis:<sup>14</sup> No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia:<sup>14</sup> No significant interference up to a triglycerides level of 1852 mg/dL.

Total protein: No significant interference from 2 to 14 g/dL total protein.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.<sup>15</sup>

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

**ACTION REQUIRED**

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

**Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.**

**Limits and ranges****Measuring range**

COBAS INTEGRA 400 plus system:  
0.61-24 µg/dL (7.9-310 nmol/L)

COBAS INTEGRA 800 system:  
0.81-24 µg/dL (10.4-310 nmol/L)

**Lower limits of measurement***Lower detection limit of the test*

COBAS INTEGRA 400 plus system:  
0.61 µg/dL (7.9 nmol/L)

COBAS INTEGRA 800 system:  
0.81 µg/dL (10.4 nmol/L)

**Total T<sub>4</sub> (Thyroxine)**

The lower detection limit represents the lowest measurable analyte level that can be distinguished from the zero calibrator at a 95 % confidence level.

**Expected values**

As total T<sub>4</sub> concentrations can be influenced by sex, geographic area, and other factors, *it is very important* that normal ranges be determined by each laboratory. As a general reference, 4 to 12 µg/dL (51.6 to 155 nmol/L) or 5 to 11 µg/dL (64.5 to 142 nmol/L) are typically stated as approximate adult normal ranges.<sup>16</sup>

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

**Specific performance data**

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

**Precision**

Precision was determined using controls in an internal protocol with repeatability (n = 20) and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained:

Repeatability	Mean		SD	CV
	µg/dL	nmol/L		
Level 1	4.5	58.1	0.18	4.1
Level 2	7.8	101	0.24	3.1
Level 3	14.9	192	0.39	2.6

Intermediate precision	Mean		SD	CV
	µg/dL	nmol/L		
Level 1	4.5	58.1	0.35	7.6
Level 2	7.8	101	0.27	3.4
Level 3	14.9	192	0.35	2.4

**Method comparison**

Total T<sub>4</sub> values for human serum samples obtained on COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Total T<sub>4</sub> (Thyroxine) reagents were compared to those determined with COBAS FP reagents on the COBAS FARA II analyzer and a commercially available FPIA method.

	COBAS FARA II analyzer	FPIA
Number of samples	194	194
Range of values	min. 0.94 µg/dL max. ≥ 24.0 µg/dL	0.94 µg/dL ≥ 24.0 µg/dL
Slope	0.987	0.943
Intercept (µg/dL)	0.695	0.520
Correlation coefficient	0.992	0.994

**Analytical specificity**

The following cross-reactive substances were evaluated on a COBAS INTEGRA 700 analyzer in normal human serum spiked with total T<sub>4</sub> at 10 µg/dL (129 nmol/L). Each substance was tested at 10 times the highest concentration for its therapeutic or normal range, as per the protocol described by NCCLS.<sup>17</sup> The precision of the assay was taken into account when determining cross-reactivity.

$$\text{Cross-reactivity (\%)} = \frac{100 \times (\text{analytical result} - \text{analyte conc.})}{\text{conc. of interferent}}$$

**Cross-reactivity**

Drug	Level tested µg/mL	Cross-reactivity %
<i>l</i> -Triiodothyronine	170	1.3

<i>d</i> -Triiodothyronine	170	0.8
Phenytoin	20 000	0
Phenylbutazone	45 000	0
Salicylate	10 000	0

In a similar study, the following structurally related or potentially co-administered compounds were tested using the COBAS INTEGRA Total T<sub>4</sub> (Thyroxine) reagents on a COBAS FARA II analyzer and normal human serum spiked with total T<sub>4</sub> at 10 µg/dL (129 nmol/L).

Drug	Level tested µg/mL	Cross-reactivity %
<i>l</i> -Diiodothyronine	170	ND
<i>l</i> -Diiodotyrosin	170	ND
<i>l</i> -Monoiodotyrosine	170	ND
Propylthiouracil	400	ND
<i>d</i> <i>l</i> -Tyrosine	100	ND

ND = Not Detectable

**References**

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# T4

## Total T4 (Thyroxine)

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

### Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

	Contents of kit
	Volume after reconstitution or mixing
	Global Trade Item Number

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Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim  
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