

## Overview

### Useful For

Determining thyroid status of sick, hospitalized patients

Determining thyroid status of patients in whom abnormal binding proteins have been identified

Possibly useful in pediatric patients

### Method Name

Equilibrium Dialysis/Tandem Mass Spectrometry (MS/MS)

### NY State Available

Yes

## Specimen

### Specimen Type

Serum

### Ordering Guidance

The routine free T4 is faster and provides useful information for most patients; order FRT4 / T4 (Thyroxine), Free, Serum.

### Necessary Information

Include name and telephone number of contact physician

### Specimen Required

**Supplies:** Sarstedt Aliquot Tube, 5 mL (T914)

**Collection Container/Tube:**

**Preferred:** Red top

**Acceptable:** Serum gel

**Submission Container/Tube:** Plastic vial

**Specimen Volume:** 2.6 mL

### Collection Instructions:

1. Collect specimen immediately before next scheduled dose.
2. Within 2 hours of collection, centrifuge, and aliquot serum into a plastic vial.

### Specimen Minimum Volume

1.2 mL

### Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	Reject

**Specimen Stability Information**

Specimen Type	Temperature	Time	Special Container
Serum	Refrigerated (preferred)	28 days	
	Frozen	21 days	
	Ambient	7 days	

**Clinical & Interpretive**
**Clinical Information**

Thyroxine (T4) and triiodothyronine (T3) are the 2 biologically active thyroid hormones. T4 makes up more than 80% of circulating thyroid hormones.

Following secretion by the thyroid gland, approximately 70% of circulating T4 and T3 are bound to thyroid-binding globulin (TBG), while 10% to 20% each are bound to transthyretin (TTR) and albumin, respectively. Less than 0.1% circulates as free T4 (FT4) or free T3 (FT3). FT4 and FT3 enter and leave cells freely by diffusion. Only the free hormones are biologically active, but bound and free fractions are in equilibrium. Equilibrium with TTR and albumin is rapid. By contrast, TBG binds thyroid hormones very tightly and equilibrium dissociation is slow. Biologically, TBG-bound thyroid hormone serves as a hormone reservoir, and T4 serves as a prohormone for T3. Within cells, T4 is either converted to T3, which is about 5 times as potent as T4, or reverse T3, which is biologically inactive. Ultimately, T3, and to a much lesser degree T4, bind to the nuclear thyroid hormone receptor, altering gene expression patterns in a tissue-specific fashion.

Under normal physiologic conditions, FT4 and FT3 exert direct and indirect negative feedback on pituitary thyrotropin (TSH) levels, the major hormone regulating thyroid gland activity. This results in tight regulation of thyroid hormone production and constant levels of FT4 and FT3 independent of the binding protein concentration. Measurement of FT4 and FT3, in conjunction with TSH measurement, therefore, represents the best method to determine thyroid function status. It also allows determination of whether hyperthyroidism (increased FT4) or hypothyroidism (low FT4) are primary (most cases; TSH altered in the opposite direction as FT4) or secondary/tertiary (hypothalamic/pituitary origin; TSH altered in the same direction as FT4). By contrast, total T4 and T3 levels can vary widely as a response to changes in binding protein levels, without any change in free thyroid hormone levels and, hence, actual thyroid function status.

FT4 is usually measured by automated analog immunoassays. In most instances, this will result in accurate results. However, abnormal types or quantities of binding proteins found in some patients and most often related to other illnesses or drug treatments, may interfere in the accurate measurement of FT4 by analog immunoassays. These problems can be overcome by measuring FT4 by equilibrium dialysis, free from interfering proteins.

**Reference Values**

0.8-2.0 ng/dL

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Reference values apply to all ages.

**Interpretation**

All free hormone assays should be combined with thyrotropin measurements.

Free thyroxine (FT4) levels below 0.8 ng/dL indicate possible hypothyroidism. FT4 levels above 2.0 ng/dL indicates possible hyperthyroidism.

Neonates can have significantly higher FT4 levels. The hypothalamic-pituitary-thyroid axis can take several days or, sometimes, weeks to mature.

**Cautions**

Certain drugs may cause short-term free thyroxine fluctuations:

- Heparin
- Salicylates
- Acetylsalicylic acid (aspirin)
- Salicylic acid (salsalate)
- Furosemide
- Fenclofenac
- Mefenamic acid
- Flufenamic acid
- Diclofenac
- Diflunisal
- Phenytoin
- Carbamazepine

**Clinical Reference**

1. De Brabandere VI, Hou P, Stockl D, Thienpont LM, De Leenheer AP. Isotope dilution-liquid chromatography/electrospray ionization-tandem mass spectrometry for the determination of serum thyroxine as a potential reference method. *Rapid Commun Mass Spectrom*. 1998;12(16):1099-1103
2. Jain R, Uy HL. Increase in serum free thyroxine levels related to intravenous heparin treatment. *Ann Intern Med*. 1996;124(1 Pt 1):74-75
3. Stockigt JR. Free thyroid hormone measurement. A critical appraisal. *Endocrinol Metab Clin North Am*. 2001;30(2):265-289
4. Sakai H, Nagao H, Sakurai M, et al. Correlation between serum levels of 3,3',5'-triiodothyronine and thyroid hormones measured by liquid chromatography-tandem mass spectrometry and immunoassay [published correction appears in *PLoS One*. 2016;11(7):e0159169]. *PLoS One*. 2015;10(10):e0138864.doi:10.1371/journal.pone.0138864
5. Kahric-Janjic N, Soldin SJ, Soldin OP, West T, Gu J, Jonklaas J. Tandem mass spectrometry improves the accuracy of free thyroxine measurements during pregnancy. *Thyroid*. 2007;17(4):303-311.doi:10.1089/thy.2006.0303

**Performance****Method Description**

The tandem mass spectrometry method utilizes an equilibrium dialysis technique to separate free thyroxine (FT4) from

serum proteins, including protein-bound T4. An isotope of thyroxine (L-thyroxine-[thyrosine-ring-(13)C6]) is added to the dialysate and serves as an internal standard. A liquid-liquid extraction with ethyl acetate further purifies the dialysate. After being dried down and reconstituted, the remaining extract is injected onto the tandem mass spectrometer where it undergoes high-throughput liquid chromatography combined with an electrospray ion source. (Unpublished Mayo method)

**PDF Report**

No

**Day(s) Performed**

Monday, Wednesday, Thursday

**Report Available**

3 to 8 days

**Specimen Retention Time**

2 weeks

**Performing Laboratory Location**

Rochester

**Fees & Codes****Fees**

- Authorized users can sign in to [Test Prices](#) for detailed fee information.
- Clients without access to Test Prices can contact [Customer Service](#) 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact [Customer Service](#).

**Test Classification**

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

**CPT Code Information**

84439

**LOINC® Information**

Test ID	Test Order Name	Order LOINC® Value
FRT4D	T4 (Thyroxine), Free by Dialysis, S	6892-4

  

Result ID	Test Result Name	Result LOINC® Value
8859	T4 (Thyroxine), Free by Dialysis, S	6892-4