# Test Definition: INSFT

Insulin, Free and Total, Serum

## Overview

### Useful For

Assessing free (bioactive) insulin concentrations in patients with known or suspected anti-insulin antibodies

### Profile Information

| Test Id | Reporting Name | Available Separately | Always Performed |
|---|---|---|---|
| INSF | Insulin, Free, S | No | Yes |
| INSTO | Insulin, Total, S | Yes, (Order INS) | Yes |

### Highlights

Patients treated with exogenous insulin preparations might develop autoantibodies against insulin.

If significant differences between the total and free insulin concentrations are detected following ultrafiltration, the presence of anti-insulin antibodies is suspected.

### Method Name

Electrochemiluminescence Immunoassay (ECLIA)

### NY State Available

Yes

## Specimen

### Specimen Type

Serum

### Specimen Required

**Patient Preparation:**

**1. Fasting: 8 hours, required**

2. For 12 hours before specimen collection, **do not** take multivitamins or dietary supplements containing biotin (vitamin B7), which is commonly found in hair, skin, and nail supplements and multivitamins.

**Collection Container/Tube:**

**Preferred:** Serum gel

**Acceptable:** Red top

**Submission Container/Tube:** Plastic vial

**Specimen Volume:** 2.45 mL

**Collection Instructions:**

1. Avoid hemolysis

2. Label specimens with corresponding collection times.
3. Serum-gel tubes should be centrifuged within 2 hours of collection.
4. Red-top tubes should be centrifuged and aliquoted within 2 hours of collection.
5. Send specimen refrigerated

**Additional Information:** If multiple specimens are collected, send separate order for each specimen.

### Specimen Minimum Volume

0.75 mL

### Reject Due To

| | |
|---|---|
| Gross hemolysis | Reject |
| Gross lipemia | OK |
| Gross icterus | OK |
| Autopsy/post mortem specimen | Reject |

### Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Serum | Refrigerated (preferred) | 14 days | |
| | Ambient | 24 hours | |
| | Frozen | 180 days | |

## Clinical & Interpretive

### Clinical Information

Insulin is produced by the beta cells of the pancreas. It regulates the uptake and utilization of glucose and is also involved in protein synthesis and triglyceride storage.

Some patients receiving insulin may develop antibodies that bind insulin. These antibodies may or may not affect the activity and metabolism of insulin.

The presence of insulin antibodies has 2 main consequences:
1. Insulin antibodies will directly bind to insulin, making it unavailable for metabolic activity.
2. Insulin antibodies may adversely affect the binding characteristics of insulin in immunoassays, making reliable quantitation difficult.

Free (bioactive) insulin could be measured after ultrafiltration to remove anti-insulin antibodies and their bound insulin. If insulin antibodies are not present, the free and total insulin concentrations should be equivalent. The laboratory will report results of the total insulin (without ultrafiltration) and the free insulin (after ultrafiltration).

## Reference Values

FREE INSULIN:
3-25 mcIU/mL

TOTAL INSULIN:
3-25 mcIU/mL

## Interpretation

Free insulin represents the portion of total insulin unbound by anti-insulin antibodies in the circulation. This fraction serves as a measure of biologically active insulin and provides an indication of the true relationship between insulin and blood glucose.

Most individuals do not have anti-insulin antibodies in circulation and therefore the free and total insulin concentrations would be equivalent.

When a significant difference between total and free insulin Concentrations is observed following ultrafiltration, the result is suggestive of the presence of insulin antibodies. In these cases, confirmation of the presence of anti-insulin antibodies (Mayo Test ID: INAB) may be helpful.

## Cautions

In rare cases, some individuals can develop antibodies to mouse or other animal antibodies (often referred to as human anti-mouse antibodies [HAMA] or heterophile antibodies), which may cause interference in some immunoassays. The presence of antibodies to streptavidin or ruthenium can also rarely occur and may also interfere in this assay. Caution should be used in interpretation of results, and the laboratory should be alerted if the result does not correlate with the clinical presentation.

Hemolysis interferes with this assay, as insulin-degrading peptidases are released from erythrocytes.This assay has 100% cross-reactivity with recombinant human insulin (Novolin R and Novolin N). It does not recognize other commonly used analogues of injectable insulin (ie, insulin lispro, insulin aspart, and insulin glargine).

## Clinical Reference

1. Lupsa BC, Chong AY, Cochran EK, Soos MA, Semple RK, Gorden P. Autoimmune forms of hypoglycemia. Medicine (Baltimore). 2009;88(3):141-153
2. Sapin R, Le Galudec V, Gasser F, Pinget M, Grucker D. Elecsys insulin assay: free insulin determination and the absence of cross-reactivity with insulin lispro. Clin Chem. 2001;47(3):602-605
3. Sacks DB: Diabetes mellitus. In: Rifai N, Horvath AR, Wittwer CT, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics 6th ed. Elsevier; 2018:1160-1200

## Performance

### Method Description

The Roche cobas insulin method is a sandwich electrochemiluminescence immunoassay that employs a biotinylated monoclonal insulin-specific antibody and a monoclonal insulin-specific antibody. Insulin in the specimen reacts with both

the biotinylated monoclonal insulin-specific antibody (mouse) and the monoclonal insulin-specific antibody (mouse) labeled with a ruthenium complex, forming a sandwich complex. Streptavidin-coated microparticles are added, and an interaction occurs between the biotin and streptavidin, binding the complex to the solid phase. The mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of voltage to the electrode induces the chemiluminescent emission, which is then measured. (Package insert: Insulin reagent, Roche Diagnostics; 2022)

For free insulin, specimen immunoglobulins and their bound insulin are filtered out using a centrifugal ultrafiltration device with a molecular weight cut-off of 125,000 daltons. The filtrate, containing free insulin alone, is analyzed using the method described above.

### PDF Report
No

### Day(s) Performed
Monday, Wednesday, Friday

### Report Available
1 to 4 days

### Specimen Retention Time
14 days

### Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

## 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 has been modified from the manufacturer's instructions. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

### CPT Code Information
83527-Free Insulin
83525-Total Insulin

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|

| | | |
|---|---|---|
| INSFT | Insulin, Free and Total, S | 48615-9 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| INSTO | Insulin, Total, S | 27873-9 |
| INSF | Insulin, Free, S | 6901-3 |