

Iron (Total) Reagent

Catalog #: 43716

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

For the *in vitro* quantitative determination of Iron in serum.

METHODOLOGY

Iron exists in serum complexed with transferrin, a transport protein. Most early procedures for Iron determination involved dissociation of the iron from the iron-protein complex, precipitation of the proteins, and then measurement of the iron content of the protein free filtrate.

Many chromagens have been used in the determination including thiocyanate o-phenanthroline, bathophenanthroline and TPTZ. In 1971, Persijn et al¹ presented a method using the chromagen ferrozine, described by Stookey². This method did not require protein precipitation and was more sensitive than previous methods. The present procedure is a modification of the Persijn method.

Principle:

Serum Iron: Transferrin-bound Iron is released at an acid pH and reduced from ferric to ferrous ions. These ions react with ferrozine to form a violet colored complex which is measured spectrophotometrically at 560nm. The absorbance measured at this wavelength is proportional to serum iron concentration.

CLINICAL SIGNIFICANCE³

In most cases, both serum Iron and TIBC values are necessary for greatest diagnostic significance. Low serum iron values are seen in chronic blood loss, insufficient intake or absorption of Iron, and increased demand on the body stores (e.g. pregnancy). Elevated serum iron values are seen in increased red cell destruction, decreased red cell synthesis, increased Iron intake, or increased Iron stores release.

Increase in the TIBC may be due to increased production of apotransferrin (e.g. chronic Iron deficiency) or an increased release of ferritin, as in hepatocellular necrosis.

Decreases in the TIBC can occur with cirrhosis and hemochromatosis due to a deficiency in ferritin, or in nephrosis due to a loss of apotransferrin.

REAGENT COMPOSITION

| Active Ingredients | Concentrations |
|--|----------------|
| IRON BUFFER REAGENT: Hydroxylamine hydrochloride in acetate buffer, pH 4.5 with surfactant. | 220mM |
| IRON COLOR REAGENT: Ferrozine in hydroxylamine hydrochloride. | 16.7mM |

Precautions

- All reagents are toxic. Do not pipette by mouth. Avoid all contact.
- This reagent is for *in vitro* diagnostic use only.

REAGENT STORAGE

Store all reagents refrigerated at 2-8°C.

REAGENT DETERIORATION

- All reagents should be clear. Turbidity may indicate contamination and the reagent should not be used.

SPECIMEN COLLECTION AND STORAGE

- Fresh, unhemolyzed serum is the specimen of choice.
- Serum should be separated as soon as clot has formed.
- Heparinized plasma may be used but other anticoagulants should not be used to avoid possible Iron contamination.⁴
- Serum Iron is reported to be stable for four days at room temperature (15-30°C) and seven days at 2-8°C.

INTERFERENCES

- Certain drugs and other substances are known to influence circulating Iron levels. See Young, et al⁵.
- Iron contained in hemoglobin does not react in this method, therefore, slight hemolysis will not interfere. However, gross hemolysis (pink or red specimens) will contribute to the absorbance measured at the wavelength used and should be avoided.³
- To make tubes, pipettes, etc. Iron free, they must be washed with dilute (1:2) hydrochloric or nitric acid, followed by several rinsing with Iron-free deionized or distilled water.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- SDI CA480 Clinical Chemistry System
- Iron-free deionized water and related equipment, e.g.: pipettes
- Analyzer specific consumables, e.g.: sample cups
- Control, and Calibrator materials such as those provided by SDI Biomed.

ASSAY PROCEDURE

System Parameters

| | |
|---------------------|----------------|
| Iron (Total) | |
| TEMPERATURE: | 37°C |
| WAVELENGTH: | 560 nm |
| DIRECTION: | Increase |
| SAMPLE / RGT RATIO: | 1 : 5 |
| e.g. Sample Vol. | 0.5 mL (500mL) |
| Reagent Vol. | 2.5 mL |
| Chromogen Vol. | 0.05 mL (50mL) |
| REACTION TIME: | 10 Min |

Calculations

A = Absorbance
STD = Standard

$$\frac{A_2 \text{ Test} - A_1 \text{ Test}}{A_2 \text{ Std} - A_1 \text{ Std}} \times \text{Conc. Of Std.} = \text{Total Iron (ug/dL)}$$

Example: $A_1 \text{ Test} = 0.08$ $A_2 \text{ Test} = 0.15$
 $A_1 \text{ Std} = 0.00$ $A_2 \text{ Std} = 0.40$

Then: $\frac{0.15 - 0.08}{0.40 - 0.00} = \frac{0.07}{0.40} = 0.175 \times 500 = 87.5 \text{ ug/dL}$

CALIBRATION

The procedure is calibrated with an aqueous iron standard (500ug/dL) or an appropriate serum calibrator.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known Iron values.

EXPECTED VALUES

Iron (Total) 60 - 150 ug/dL

It is strongly recommended that each laboratory determine the normal range for its particular population.

PERFORMANCE

Linearity:

When run as recommended the assay is linear to 500 ug/dL.

Samples with values above 500 ug/dL must be diluted 1:1 with normal saline, re-assayed and result multiplied by two.

Method Comparison:

Studies performed between this procedure and a similar Serum Iron procedure yielded the following results:

| | |
|--------------------------|--------------|
| Correlation Coefficient: | 0.983 |
| Slope: | 0.79 |
| Intercept: (mg/dL) | 0.13 (mg/dL) |

Precision:

| Within Run | Level 1 | Level 2 | Level 3 |
|--------------|---------|---------|---------|
| Mean (mg/dL) | 110 | 184 | 251 |
| S.D. (mg/dL) | 3.0 | 3.1 | 3.3 |
| C.V. (%) | 2.7 | 1.7 | 1.3 |

Run to Run

| | | | |
|--------------|-----|-----|-----|
| Mean (mg/dL) | 109 | 177 | 249 |
| S.D. (mg/dL) | 5.1 | 3.8 | 4.5 |
| C.V. (%) | 4.7 | 2.1 | 1.8 |

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Manufactured for:



23679 Calabasas Road, Unit 241
Calabasas, CA 91302
800-952-2470

PI: 43716.01

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