IRON

(LIQUID)

3 x 60, 3 x 12 ml

RE – ORDER IRN1150

INTENDED USE:

This set of reagents is intended for the quantitative in vitro measurement of iron in serum.

CLINICAL SIGNIFICANCE:

Iron in the body is a necessary metal required not only for the synthesis of hemoglobin but also for many cellular enzymes and coenzymes. Iron is transported in serum bound to the protein transferrin. Normally, only about one-third of the available binding sites on transferrin are occupied by iron. The total iron binding capacity in serum therefore, includes the amount of iron already bound to the transferrin (serum iron) plus the amount of iron required to saturate the unoccupied binding sites of transferrin.

Clinically the determination of serum iron and total iron binding capacity is useful in the differential diagnosis of anemias and other iron disorders (1).

TEST SUMMARY:

The spectrophotometric measurement of serum iron is accomplished by releasing the protein bound iron from its carrier protein transferrin and complexing the released iron with a suitable chromogen.

In our method the serum sample is added to an acidic buffered reagent containing hydroxylamine, thiourea and Ferene®.

The acid pH of the buffered reagent releases the iron which is in the ferric form from the transferrin (2). The released ferric iron is then reduced to the ferrous form by hydroxylamine. This ferrous iron reacts with Ferene to produce a colored complex. The absorbance of this colored complex, read at 595 nm, is proportional to the concentration of iron in the sample. The thiourea in the buffered reagent effectively suppresses any interference from copper (3).

REAGENT COMPOSITION:

Serum Iron Buffer	
Reactive ingredients:	
Hydroxylamine Hydrochloride	216 mmol/L
Thiourea	26 mmol/L
Non-reactive ingredients:	
Buffers, stabilizers and preservatives	
Chromogen	
Reactive ingredients:	
Ferene	33 mmol/L
Iron Standard	
Reactive ingredients:	
Iron	500 µg/dL
Hydroxylamine Hydrochloride	719 mmol/L

REAGENT PREPARATION:

Prepare the Serum Iron Color Reagent by mixing 1 mL of Iron/ TIBC Chromogen (Cat. No. 85166) with 50 mL of Serum Iron Buffer (Cat. No. 85164). This color reagent is stable for 6 weeks stored at 2–8 °C. Store in amber container and protect from light. Bring Color Reagent to room temperature prior to use.

REAGENT STORAGE AND STABILITY:

The reagents as supplied are stable at 2–8 °C until the expiration date on the bottle label. The prepared color reagent is stable for 6 weeks at 2–8 °C. Mix and store the color reagent in amber container free of iron contamination; protect from light (see Reagent Preparation, above).

PRECAUTIONS:

Harmful by inhalation, in contact with skin, and if swallowed. Possible risks of irreversible effects.

Avoid contact with skin, wash immediately with plenty of water for at least 10 minutes.

Irritating to eyes and skin.

Avoid contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water for at least 10 minutes. If swallowed, seek medical advice immediately and show this container or label.

Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

SPECIMEN COLLECTION AND STORAGE:

Use only serum samples, free of hemolysis, separated from the clot as soon as possible. DO NOT use hemolyzed samples. DO NOT use heparinized plasma. Use only iron-free tubes and syringes to collect blood samples. Serum iron is stable for at least 4 days at 18–25 °C and 7 days at 0–5 °C (4).

INTERFERING SUBSTANCES:

Drugs and other substances that interfere with the determination of iron have been reported by Young (5, 6).

MATERIALS REQUIRED BUT NOT PROVIDED:

Spectrophotometer or colorimeter capable of measuring absorbance at 595 nm.

Matched cuvettes, preferably with 1 cm light path.

Pipettes to measure reagents and samples.

Distilled or deionized water.

Constant temperature water bath. Temperature is not critical but should be constant.

MATERIALS PROVIDED:

Reagents for the quantitative measurement of serum iron.

IRN1150 3X60, 3X12 with standard

TEST PROCEDURE:

Bring Iron Color Reagent to room temperature prior to performing assay.

Set up assay as follows:

Water	Sample		Standard			
Blank	Test	Blank	Test	Blank	Test	
Iron Buffer	2 mL	–	2 mL	–	2 mL	–
Iron Color Reagent	–	2 mL	–	2 mL	–	2 mL
Water	0.4 mL	0.4 mL	–	–	–	–
Standard	–	–	–	–	0.4 mL	0.4 mL
Sample	–	–	0.4 mL	0.4 mL	–	–

Mix. Incubate tests and blanks at 37 °C for 10 minutes.

Set instrument to 595 nm and adjust to zero absorbance with distilled or deionized water.

Read and record the absorbance of each test and its respective blank at 595 nm. Subtract the absorbance of each blank from the absorbance of its respective test. This is the corrected absorbance (A_c) for each test: water, sample and standard.

$A_c = A_{test} - A_{blank}$

CALIBRATION:

This assay requires the use of an iron standard which is an integral part of the assay. Use only the iron standard provided with this reagent as a calibrating standard. The use of other iron standards may not produce accurate results.

QUALITY CONTROL:

Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and technique. Commercially available control material with established values for serum iron and TIBC concentrations may be used. Raichem Assayed Control Serum, Level 1 (Cat. No. 83082) and Level 2 (Cat. No. 83083) are recommended for this purpose.

CALCULATIONS:

(A_c sample) - (A_c water)

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$\frac{\text{(A standard) - (A water)}}{\text{(A standard) - (A water)}} \times C_{Std}$ = serum iron in µg/dL.

where C standard is the concentration of the standard in µg/dL.

Sample Calculation:

A_c water = 0.010

A_c sample = 0.098

A_c standard = 0.525 for 500 µg/dL iron

$\text{Then: } \frac{0.098 - 0.010}{0.525 - 0.010} \times 500 = 85$ µg/dL

LIMITATIONS OF THE PROCEDURE:

Samples with serum iron concentrations higher than 500 µg/dL should be diluted with iron-free water and the assay repeated. Multiply the results by the dilution used.

The use of incubation temperatures lower than 37 °C will require an incubation time longer than 10 minutes for the reactions to reach completion. Ensure that the reaction has reached completion at the selected temperature.

REAGENT PERFORMANCE:

Linearity: The assay is linear to 500 µg/dL.

Correlation: Results obtained with this reagent for serum iron were compared with those obtained using a similar commercial reagent (Sigma Chemical Company). Fifty-seven serum samples were assayed ranging in serum iron concentrations from 11 µg/dL to 246 µg/dL. The correlation coefficient was 0.981 and the regression equation was y = 0.96x + 8.81.

Precision:

Within Run				Total		
Mean (µg/dL)	96	122	242	92	122	239
SD (µg/dL)	2.50	1.33	3.65	2.90	6.00	4.26
CV (%)	2.6	1.0	1.5	3.1	4.9	1.8
N	11	11	11	10	10	10

REFERENCE RANGE:

Iron, Total = 60 – 150 ug/dl

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

REFERENCES:

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