

# Iron Reagent / UIBC Reagent

## Ferene Method

### PRODUCT SUMMARY

Stability	:	Until expiry at 2-8°C
Linear Range - Iron	:	5 - 1000 µg/dL (0.9 - 179 µmol/L)
Linear Range - UIBC	:	Up to 500 µg/dL (90 µmol/L)
Specimen Type	:	Serum
Method	:	Endpoint
Reagent Preparation	:	Supplied ready to use.

**IVD**

### SYMBOLS IN PRODUCT LABELLING

<b>EC REP</b>	Authorized Representative		Temperature Limitation
<b>IVD</b>	For in vitro diagnostic use		Use by/Expiration Date
<b>LOT</b>	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
<b>REF</b>	Catalogue number		Manufactured by
	Consult instructions for use		Xi - Irritant
<b>REAG R1</b>	Iron Buffer	<b>REAG R1B</b>	UIBC Binding Reagent
<b>REAG R1A</b>	UIBC Buffer	<b>STD</b>	Iron Standard
<b>REAG R2</b>	Iron Color Reagent		

### IRON REAGENT

#### INTENDED USE

In vitro diagnostic use for the quantitative determination of iron in serum.

#### SUMMARY AND EXPLANATION

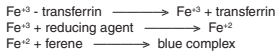
Iron is essential for the existence of most living organisms. In the body iron is present in various enzymes involved in oxidative mechanisms. Iron is the major constituent of hemoglobin and myoglobin and is involved in the transport of oxygen to tissues. It is stored in the body by the iron containing protein, ferritin, and transported by the protein transferrin.<sup>1</sup>

Serum iron is that iron, present as the Fe<sup>3+</sup> form, which is associated with transferrin and does not include iron associated with other iron, containing proteins. Serum iron concentration is decreased in patients with iron deficiency anemia as a result of insufficient intake of dietary iron or inadequate iron absorption. Decreased levels are also seen in patients presenting with acute inflammation, abscess, immunizations, and myocardial infarction. Low serum iron occurs in individuals who have recently donated blood. Serum iron also decreases during menstruation. Moderate depression of serum iron can occur in individuals with malignancies or rheumatoid arthritis.<sup>1,2</sup>

Increased serum iron occurs as a result of ingestion of iron-fortified vitamins, acute hepatitis, acute iron poisoning in children, and in iron loading disorders such as hereditary hemochromatosis and sideroblastic anemia. The use of oral contraceptives raises serum iron levels; however, upon discontinued use of contraceptives, serum iron decreases as much as 30% as a result of uterine bleeding.<sup>2</sup>

There are many methods available for determining serum iron. All colorimetric procedures utilize a reaction in which serum iron is released from transferrin by reduction in pH of the serum. A chromogen is then reacted with the reduced iron to produce an iron-chromogen complex. Complexing agents in common use include sulfonated bathophenanthroline, tripyridyl triazine (TPTZ), ferrozine, terosite<sup>1</sup>, and ferene<sup>3</sup>. The resulting Fe-chromogen complex has a visible range spectrum with peaks in the 500-600 nm range dependent upon the complexing agent used.

#### PRINCIPLE



The Thermo Iron Reagent utilizes the chromogen ferene to bind ferrous ions. Iron is released from transferrin by the addition of an acidic buffer. Fe<sup>3+</sup> is then reduced to Fe<sup>2+</sup> by a reducing agent. The ferrous ions react with ferene dye to form a blue complex which is measured spectrophotometrically and is proportional to the amount of iron present.

#### REAGENT COMPOSITION

Active Ingredient	Concentration
<b>Iron Buffer (R1)</b>	
Hydroxylamine HCl	0.216 mol/L
Buffer	
Surfactant	
<b>Iron Color Reagent (R2)</b>	
Ferene	4.0 mmol/L
Buffer	
Surfactant	
<b>Iron Standard (STD)</b>	
Iron	500 µg/dL
Hydroxylamine HCl	720 mmol/L

**WARNING:** Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. For further information consult the Iron Reagent/UIBC Reagent Material Safety Data Sheet.

#### Iron Buffer (R1) and Iron Standard (STD)

R43 May cause sensitisation by skin contact.  
S24 Avoid contact with skin.  
S37 Wear suitable gloves.

California state regulations require the following precaution for this product.  
**WARNING:** This product contains a chemical known to the State of California to cause cancer.

#### REAGENT PREPARATION

Reagents and Standard are ready to use as supplied.

#### STORAGE AND STABILITY

The reagents and standard are stable until the expiration date stated on the label when stored at 2-8°C. After opening and use, close cap tightly when storing.

#### DETERIORATION

- The Iron Buffer (R1) should be a clear, colorless solution.
- The Iron Color Reagent (R2) should be a clear, pale yellow solution.
- The Iron Standard (STD) should be a clear, colorless solution.
- Failure to achieve assay values on freshly prepared control sera could indicate deterioration.

#### SPECIMEN COLLECTION

- Non-hemolyzed serum is the recommended sample.<sup>4</sup>
- Specimens should be collected in the morning in order to avoid low results due to diurnal variation.<sup>4</sup>
- It is recommended that specimens be collected and processed as described in NCCLS<sup>5</sup> (H3, H4, H18, M29-T2) or equivalent publications.

#### INTERFERING SUBSTANCES

- At approximately 225 µg/dL (40.3 µmol/L), iron, there is no interference from hemoglobin up to 317 mg/dL. At approximately 75 µg/dL (13.4 µmol/L) iron, there is no interference from hemoglobin up to 162 mg/dL.
- Bilirubin up to 23 mg/dL (393 µmol/L) does not cause significant interference with this assay.
- At approximately 225 µg/dL (40.3 µmol/L) iron, there is no interference from lipemia up to 400 mg/dL (4.6 mmol/L) triglyceride. At approximately 75 µg/dL (13.4 µmol/L) iron, there is no interference from lipemia up to 226 mg/dL (2.6 mmol/L) triglyceride.
- Young has reviewed drug effects on serum iron levels.<sup>7</sup>

#### SAMPLE STORAGE

Iron in serum (after separation from red cells) is stable for 4 days at room temperature or 7 days at 2-8°C.<sup>8</sup>

#### MATERIALS PROVIDED

- Iron Buffer (R1)
- Iron Color Reagent (R2)
- Iron Standard (STD)

#### MATERIALS REQUIRED BUT NOT PROVIDED

- Cobas Mira<sup>®</sup> Analyzer, or equivalent, with Manual and Accessories.
- Normal and abnormal assayed control material.

#### STABILITY OF FINAL REACTION MIXTURE

The instrument automatically computes every determination at the same time interval.

#### CALIBRATION

It is not necessary to determine a standard curve with this procedure since the reaction is linear to 1000 µg/dL (179 µmol/L). The Iron Standard included in this kit is traceable to NIST Reference Material. For calibration frequency on automated instruments, refer to the instrument manufacturers specifications.

#### LINEARITY

Linearity extends from 5 to 1000 µg/dL (0.9 - 179 µmol/L). Samples exceeding linearity should be diluted with normal saline and repeated. Multiply the concentration by the dilution factor when calculating the unknown.

#### QUALITY CONTROL

Normal and abnormal control sera of known concentrations of iron should be analyzed routinely with each group of unknown samples.

#### CALCULATION OF RESULTS

Results, expressed as µg/dL, are automatically calculated. TIBC may be calculated from Serum Iron and UIBC levels as follows:

$$\text{TIBC} = \text{Serum Iron} + \text{UIBC}$$

% Saturation may be calculated from serum Iron and TIBC levels as follows:

$$\% \text{ Saturation} = \frac{\text{Iron}}{\text{TIBC}} \times 100$$

The adult normal range for % Saturation is 20% - 55%.<sup>8</sup>

#### LIMITATIONS

See Storage and Stability, Deterioration, Specimen Collection, Interfering Substances, Sample Storage, Stability of Final Reaction Mixture, and Linearity sections for limitations to this procedure.

#### EXPECTED VALUES

A reference range of 65 - 175 µg/dL (11.6 - 31.3 µmol/L) for males and 50 - 170 µg/dL (9.0 - 30.4 µmol/L) for females has been reported in the literature.<sup>6</sup> These ranges should serve only as guidelines.

It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

#### PERFORMANCE CHARACTERISTICS

The Performance Characteristics were established on a Cobas Mira analyzer. The user should establish performance characteristics if the product is utilized on another instrument.

#### PRECISION

Within-run reproducibility was established by assaying three levels of control sera 20 times.

WITHIN-RUN (µg/dL) / (µmol/L)	MEAN	STD. DEV.	CV%
Level 1	72 / 12.9	1.98 / 0.35	2.75
Level 2	195 / 34.9	0.95 / 0.17	0.49
Level 3	665 / 119.0	3.71 / 0.66	0.56

Run-to-run reproducibility was established by assaying three levels of control sera for 10 runs.

RUN-TO-RUN (µg/dL) / (µmol/L)	MEAN	STD. DEV.	CV%
Level 1	68 / 12.2	3.75 / 0.67	5.51
Level 2	191 / 34.2	4.38 / 0.78	2.29
Level 3	662 / 118.5	6.84 / 1.22	1.03

#### COMPARISON

A comparison of the Thermo Iron Reagent (y) with a commercial reagent of the same method (x) was performed on 217 serum samples in a range of 6 - 960 µg/dL (1.1 - 172 µmol/L). A correlation coefficient of 0.998 was obtained; the linear regression equation was y = 1.043x - 0.809.

#### SENSITIVITY

Based on an instrument resolution of A = 0.001, this Thermo Iron Reagent has a sensitivity of 3.2 µg/dL (0.6 µmol/L). The analytical sensitivity is 5.0 µg/dL (0.9 µmol/L). The limit of sensitivity may be a function of the analyzer. Users should establish their own sensitivity limits.

### UIBC REAGENT (UNSATURATED IRON BINDING CAPACITY)

#### INTENDED USE

In vitro diagnostic use for the quantitative determination of unsaturated iron binding capacity (UIBC) in serum.

#### SUMMARY AND EXPLANATION

Iron is essential for the existence of most living organisms. In the body iron is present in various enzymes involved in oxidative mechanisms. Iron is the major constituent of hemoglobin and myoglobin and is involved in the transport of oxygen to tissues. It is stored in the body by the iron containing protein, ferritin, and transported by the protein transferrin.<sup>1</sup> Normally about one-third of the available binding sites on transferrin are occupied by iron, therefore serum transferrin has considerable reserve iron binding capacity.<sup>2</sup> This is called serum unsaturated iron binding capacity (UIBC). Total Iron Binding Capacity (TIBC) is a measurement of the maximum concentration of iron that serum proteins, mainly transferrin, can bind. TIBC may be calculated from serum iron and UIBC levels as follows: TIBC = Serum Iron + UIBC. Serum TIBC is often increased in iron deficiency and decreased in chronic inflammatory disorders, malignancies, and in hemochromatosis.<sup>2</sup>

There are many methods available for determining unsaturated iron binding capacity. The colorimetric procedure utilizes addition of sufficient Fe<sup>2+</sup> at alkaline pH to saturate the available binding sites on transferrin. The remaining unbound Fe<sup>2+</sup> is reacted with a chromogen to produce an iron-chromogen complex. Complexing agents in common use include sulfonated bathophenanthroline, tripyridyl triazine (TPTZ), ferrozine, terosite<sup>1</sup>, and ferene<sup>3</sup>. The resulting iron-chromogen complex has a visible range spectrum with peaks in the 500 to 600 nm range dependent upon the complexing agent used.

#### PRINCIPLE



The Thermo UIBC Reagent utilizes the chromogen ferene to bind with excess ferrous ions. Iron is used to saturate transferrin at alkaline pH. The excess ferrous ions then react with ferene dye to form a blue complex which is measured spectrophotometrically and is proportional to the amount of available iron binding sites on transferrin.

#### REAGENT COMPOSITION

Active Ingredient	Concentration
<b>UIBC Buffer (R1A)</b>	
Tris	0.5 mol/L
Thiourea	26.3 mmol/L
Sodium azide	0.05%
Surfactant	
<b>UIBC Binding Reagent (R1B)</b>	
Ferrous ammonium sulfate	0.089 mmol/L
Hydroxylamine HCl	720 mmol/L
<b>Iron Color Reagent (R2)</b>	
Ferene	4.0 mmol/L
Buffer	
Surfactant	

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**WARNING:** Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Reagent contains Sodium Azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the Iron Reagent/UIBC Reagent Material Safety Data Sheet.

**UIBC Binding Reagent (R1B)**

R43 May cause sensitisation by skin contact.  
S24 Avoid contact with skin.  
S37 Wear suitable gloves.

California state regulations require the following precaution for this product.  
**WARNING:** This product contains a chemical known to the State of California to cause cancer.

**REAGENT PREPARATION**

Iron Color Reagent (R2) is ready to use as supplied. A working reagent is made from UIBC Buffer (R1A) and UIBC Binding Reagent (R1B) by mixing 10 volumes of UIBC Buffer (R1A) with 1 volume of UIBC Binding Reagent (R1B).

**STORAGE AND STABILITY**

The reagents and standard are stable until the expiration date stated on the label when stored at 2-8°C. The working reagent made by mixing UIBC Buffer (R1A) and UIBC Binding Reagent (R1B) is stable for 60 days when stored at 2-8°C. After opening and use, close cap tightly for storage.

**DETERIORATION**

- The UIBC Buffer (R1A) and UIBC Binding Reagent (R1B) should be clear, colorless solutions.
- The Iron Color Reagent (R2) should be a clear, yellow solution.
- Failure to achieve assay values on freshly prepared control sera could indicate deterioration.

**SPECIMEN COLLECTION**

- Non-hemolyzed serum is the recommended sample.<sup>4</sup>
- Specimens should be collected in the morning in order to avoid low results due to diurnal variation.<sup>4</sup>
- It is recommended that specimens be collected and processed as described in NCCLS<sup>5</sup> (H3, H4, H18, M29-T2) or equivalent publications.

**INTERFERING SUBSTANCES**

- At approximately 127 µg/dL (22.7 µmol/L) UIBC, there is no interference from hemoglobin up to 465 mg/dL. At approximately 256 µg/dL (45.8 µmol/L) UIBC, there is no interference from hemoglobin up to 887 mg/dL.
- Bilirubin up to 12.7 mg/dL (217 µmol/L) does not cause significant interference at UIBC levels of 130 µg/dL (23.3 µmol/L). Bilirubin up to 27.3 mg/dL (467 µmol/L) does not cause significant interference at UIBC levels of 278 µg/dL (49.8 µmol/L).
- Triglyceride up to 1125 mg/dL (12.8 mmol/L) does not interfere with this assay.
- Young has reviewed drug effects on serum iron levels.<sup>7</sup>

**SAMPLE STORAGE**

Iron in serum (after separation from red cells) is stable for 4 days at room temperature or 7 days at 2-8°C.<sup>6</sup>

**MATERIALS PROVIDED**

- UIBC Buffer (R1A)
- UIBC Binding Reagent (R1B)
- Iron Color Reagent (R2)

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Cobas Mira Analyzer, or equivalent, with Manual and Accessories.
- Normal and abnormal assayed control material.
- Calibrator or a suitable aqueous UIBC standard.

**STABILITY OF FINAL REACTION MIXTURE**

The instrument automatically computes every determination at the same time interval.

**CALIBRATION**

It is not necessary to determine a standard curve with this procedure since the reaction is linear to 500 µg/dL (90 µmol/L). A standard and reagent blank should be determined with each set of unknowns assayed. An aqueous standard or serum based calibrator, with an assigned value traceable to a primary standard (eg NIST or IRMM) is recommended. For calibration frequency on automated instruments, refer to the instrument manufacturers specifications.

**LINEARITY**

Linearity extends to 500 µg/dL (90 µmol/L). Samples exceeding linearity should be diluted with normal saline and repeated. Multiply the concentration by the dilution factor when calculating the unknown.

**QUALITY CONTROL**

Normal and abnormal control sera of known concentrations of unsaturated iron binding capacity should be analyzed routinely with each group of unknown samples.

**CALCULATION OF RESULTS**

Results, expressed as µg/dL, are automatically calculated. TIBC can be calculated from Serum Iron and UIBC levels as follows:

$$TIBC = \text{Serum Iron} + \text{UIBC}$$

% Saturation may be calculated from serum Iron and TIBC levels as follows:

$$\% \text{ Saturation} = \frac{\text{Iron}}{\text{TIBC}} \times 100$$

The adult normal range for % Saturation is 20% - 55%.<sup>8</sup>

**LIMITATIONS**

See Storage and Stability, Deterioration, Specimen Collection, Interfering Substances, Sample Storage, and Linearity sections for limitations to this procedure.

**EXPECTED VALUES**

A reference range of 70 - 380 µg/dL (12.5 - 68 µmol/L) for males and 70 - 390 µg/dL (12.5 - 70 µmol/L) for females was calculated from the serum iron and TIBC ranges reported in the literature.<sup>9</sup>

These ranges should serve only as guidelines. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

**PERFORMANCE CHARACTERISTICS**

The Performance Characteristics were established on a Cobas Mira analyzer. The user should establish performance characteristics if the product is utilized on another instrument.

**PRECISION**

Within-run reproducibility was established by assaying three levels of control sera 20 times.

WITHIN-RUN (µg/dL) / (µmol/L)	MEAN	STD. DEV.	CV%
Level 1	84 / 15.0	4.66 / 0.83	5.55
Level 2	235 / 42.1	8.76 / 1.57	3.73
Level 3	368 / 65.9	8.67 / 1.55	2.36

Run-to-run reproducibility was established by assaying three levels of control sera for 10 runs.

WITHIN-RUN (µg/dL) / (µmol/L)	MEAN	STD. DEV.	CV%
Level 1	88 / 15.7	6.46 / 1.16	7.34
Level 2	220 / 39.4	8.31 / 1.49	3.78
Level 3	355 / 63.5	16.55 / 2.96	4.66

**COMPARISON**

A comparison of the Thermo UIBC Reagent (y) with a commercial reagent of the same method (x) was performed on 119 serum samples in a range of 36 - 394 µg/dL (6.4 - 70.5 µmol/L). A correlation coefficient of 0.984 was obtained; the linear regression equation was y = 1.11x + 3.59.

**SENSITIVITY**

Based on an instrument resolution of A = 0.001, this Thermo UIBC Reagent has a sensitivity of 7.9 µg/dL (1.4 µmol/L). The analytical sensitivity is 10.0 µg/dL (1.8 µmol/L). The limit of sensitivity may be a function of the analyzer. Users should establish their own sensitivity limits.

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**PROCEDURE - IRON REAGENT  
Test Parameters (Cobas Mira®)**

GENERAL		Convers. Factor	1.00000
Measurement Mode	Absorb	Offset	0.00000
Reaction Mode	R-S-SR1	Test Range	Low 5.0
Calibration Mode	Calibrator		High 1000
Reagent Blank	Reag/Dil	Norm. Range	Low 27
Cleaner	Before		High 144
Wavelength	600 nm	No. of Steps	1
Decimal Position	0	Calc.Step A	Endpoint
Unit	µg/dL	Readings	First 3
ANALYSIS		Last	28
Sample Dil Name	H2O	Reaction Limit	No
Post Dil Factor	No	CALIBRATION	
Conc Factor	No	Calib. Interval	On Request
Sample	Cycle 1	Reagent Blank	
Volume	30.0 µL	Reag. Range	Low -0.1000
Diluent	10.0 µL		High 0.4000
Reagent	Cycle 1	Blank Range	Low -0.0100
Volume	270 µL		High 0.0200
Start Reagent 1	Cycle 4	Calib. Cup Pos	User Defined
Volume	20.0 µL	Cal-1	500
Diluent	10.0 µL	Replicate	2
CALCULATION		Deviation	10%
Sample Limit	No	CS1 Cup Pos	User Defined
Reac. Direction	Increase	CS2 Cup Pos	User Defined
Check	On	CS3 Cup Pos	User Defined
<b>PROCEDURE - UIBC REAGENT Test Parameters (Cobas Mira®)</b>			

GENERAL		Convers. Factor	1.00000
Measurement Mode	Absorb	Offset	0.00000
Reaction Mode	R-S-SR1	Test Range	Low 10.0
Calibration Mode	Calibrator		High 500
Reagent Blank	Reag/Dil	Norm. Range	Low 77
Cleaner	Before		High 420
Wavelength	600 nm	No. of Steps	1
Decimal Position	0	Calc.Step A	Endpoint
Unit	µg/dL	Readings	First 8
ANALYSIS		Last	32
Sample Dil Name	H2O	Reaction Limit	No
Post Dil Factor	No	CALIBRATION	
Conc Factor	No	Calib. Interval	On Request
Sample	Cycle 1	Reagent Blank	
Volume	10.0 µL	Reag. Range	Low -0.1000
Diluent	10.0 µL		High 2.0000
Reagent	Cycle 1	Blank Range	Low 0.0500
Volume	200 µL		High 0.1500
Start Reagent 1	Cycle 10	Calib. Cup Pos	User Defined
Volume	20.0 µL	Cal-1	User Defined
Diluent	10.0 µL	Replicate	2
CALCULATION		Deviation	10%
Sample Limit	No	CS1 Cup Pos	User Defined
Reac. Direction	Decrease	CS2 Cup Pos	User Defined
Check	On	CS3 Cup Pos	User Defined

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**REF**

**Reorder Information**

	REAG R1	REAG R2	REAG R1A	REAG R1B	STD
<b>Iron</b>					
1580-300	1 x 250 mL	1 x 60 mL			1 x 15 mL
<b>Iron/UIBC</b>					
1590-500	1 x 250 mL	1 x 125 mL	1 x 200 mL	1 x 20 mL	1 x 15 mL