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IRON (SFBC) Bathophenanthrolin

Reagent for quantitative determination of iron in human serum or plasma.

REF 80008 **R1** 1 x 100 mL **R2** 1 x 100 mL **R3** 1 x 10 mL

CE

IVD **IN VITRO DIAGNOSTIC USE**

CLINICAL SIGNIFICANCE (1)

Serum iron concentration connotes the Fe^{3+} bound to the serum transferrin and does not include the iron contained in serum as free hemoglobin. Serum iron concentration is decreased in many but not all patients with iron deficiency anemia and in chronic inflammatory disorders such as infection, immunization, and myocardial infarction. Greater than normal concentrations of serum iron occur in iron loading disorders such as hemochromatosis, in acute iron poisoning in children, and after oral ingestion of iron medication or parenteral iron administration or acute hepatitis.

PRINCIPLE (4)

After dissociation of iron-transferrin bound and deproteinisation by hydrochloric acid and trichloroacetic acid (TCA), the iron Fe^{3+} is reduced by thioglycolic acid in iron Fe^{2+}.
Then, ferrous iron forms with disulfonated bathophenantrolin a coloured complex which absorbance measured at 535 nm is directly proportional to the amount of iron in the specimen.

REAGENTS

Vial R1 **DEPROTEINISATION REAGENT** (Highly corrosive)

Hydrochloric acid	≥ 1.7	mol/L
Trichloroacetic acid	0.6	mol/L
Thioglycolic acid	0.43	mol/L

C, Xn: Corrosive, Harmful
R23/24/25: Toxic by inhalation, in contact with skin and if swallowed
R34: Causes burns, R37: Irritating to respiratory system
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
S38: In case of insufficient ventilation wear suitable respiratory equipment

Vial R2 **CHROMOGEN REAGENT**

Disulfonated Bathophenanthrolin	≥ 0.46	mmol/L
Sodium acetate	2	mol/L

Vial R3 **STANDARD**

Iron 200 µg/dL (35.8 µmol/L)

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.
As reagent R1 is highly corrosive, it should be handled very carefully:
- Verify the integrity of the contents before use
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

Reagents are ready for use.

STABILITY AND STORAGE

Store at 18-25°C, well cap in the original vial and away from light.
- Standard stability (vial R3): Transfer the requested quantity, recap and store at 18-25°C.
- Free from contamination, reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Discard any reagent if cloudy or if reagent blank measured at 535 nm is > 0.100.

SPECIMEN COLLECTION AND HANDLING

Serum. Unhemolysed morning specimen. Draw blood before other specimens that require anticoagulants. Do not use EDTA, oxalate or citrate.

Serum iron is stable for:
- 4 days at room temperature.
- 1 week at 2-8°C.

INTERFERENCES (3) (5)

Hemoglobin:	Positive interference.
EDTA:	Negative interference.
Total bilirubin:	No interference.
Direct bilirubin:	No interference.

Use carefully cleaned material with hydrochloric acid 0.1 N and well rinsed with demineralised water. Give a special care to the quality of water, reagents and/or specimens.

Iron medications affect serum levels for up to 2-4 weeks following administration.
For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.

CALIBRATION (6)

- Standard enclosed in the Kit (vial R3) or BIOLABO-Multicalibrator REF 95015 traceable to SRM 3126a.
- Or any calibrator traceable to a reference material or method.

The calibration frequency depends on proper instrument functions and on the preservation of reagent.

It is recommended to calibrate in the following cases:
1. When using a new batch of reagent.
2. After maintenance operations on the instrument.
1. When control values are out of range, even after using a new vial of fresh serum.

QUALITY CONTROL

- BIOLABO EXATROL-N Level I REF 95010.
- BIOLABO EXATROL-P Level II REF 95011.
- Assayed control sera referring to the same method.
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of indicated range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

Iron	µg/dL	[µmol/L]
New born	100-250	[17.9-44.8]
Infant	40-100	[7.2-17.9]
Children	50-120	[9.0-21.5]
Man	65-175	[11.6-31.3]
Woman	50-170	[9.0-30.4]

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCE CHARACTERISTICS

Within run N = 22	*Low level*	*High level*
Mean µg/dL	135	194
S.D. µg/dL	2.20	3.67
C.V. %	1.63	1.89

Between run N = 20	*Low level*	*High level*
Mean µg/dL	133.8	187.2
S.D. µg/dL	4.01	5.39
C.V. %	3.0	2.88

Detection limit: approximately 10 µg/dL (1.79 µmol/L)

Sensitivity for 200 µg/dL: approximately 0.200 Abs. at 535 nm.

LINEARITY

The assay is linear up to at least 1000 µg/dL (179 µmol/L).

Above, dilute the specimen with saline solution and reassay taking into account dilution factor. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

1- Preparation of the supernatant

Pipette into centrifuge tubes	
Specimen	1 mL
Reagent R1	1 mL
Cap and mix vigorously with an agitator (i.e. VORTEX) during 1 minute. Let stand for 5 minutes and centrifuge for 10 minutes at 3000 RPM	

2- Assay

Pipette in test tubes	**Blank**	**Standard**	**Assay**
Supernatant			1 mL
Reagent R1	0.5 mL	0.5 mL	
Demineralised water	0.5 mL		
Standard		0.5 mL	
Reagent R2	1 mL	1 mL	1 mL
Mix. Incubate for 5 minutes at room temperature. Read absorbances at 535 nm (520-550) against reagent blank. Colour is stable for 1 hour.			

Notes:

1- Volumes can be reduced proportionally.
2- This method is not suitable for automated instrument.

CALCULATION

Calculate the result as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1698-1704.*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 634-639*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-361 to 3-364*
(4) *Soc. Française Biol. Clin. Comité de standardisation Ann. Biol. Clin. 1977, 35, 275*
(5) *A systematic evaluation of bathophenantroline, ferozine and ferene in an ICSH-based method for the measurement of serum iron. D.P.DERMAN, A. GREEN, TH. BOTHWELL, B. GRAHAM, L. MC. NAMARA, A.P. Mac PHAIL and RD BAYNES Ann Clin. Biochem. 1989; 26 p.144-147*
(6) *SRM: Standard Reference Material ®*

Manufacturer | Use by | In vitro diagnostic | Temperature limitation | Catalogue number | See insert | Batch number | Store away from light | sufficient for | dilute with