BIOLABO
www.biolabo.fr
MANUFACTURER:
BIOLABO SAS,
Les Hautes Rives
02160, Maizy, France

TECHNICAL SUPPORT AND ORDERS
Tel: (33) 03 23 25 15 50
support@biolabo.fr
Latest revision: www.biolabo.fr

IRON Direct Method (Ferene)

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

REF 92108 **R1** 2 x 125 mL **R2** 1 x 10 mL **R3** 1 x 10 mL

CE

IVD

Made In France

I: corresponds to significant modifications

I INTENDED USE

This reagent is designated for professional use in laboratory (semi-automated or automated method).
It allows quantitative determination of iron in human serum and plasma.

GENERALITIES (1)

Serum iron concentration connotes the Fe^{3+} bound to the serum transferrin and does not include the iron contained in serum as free haemoglobin. 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 in acid medium, ascorbic acid reduces Fe3+ iron into Fe2+ iron. Fe2+ iron then form a coloured complex with 3 -(2-Pyridyl) -5, -6-difuryl-1, -2, -4-triazine-disulfonate (Ferene). The absorbance thus measured at 600 nm (580-620) is directly proportional to the amount of iron in the specimen. Thiourea is added in the reagent to prevent the copper interference.

REAGENTS COMPOSITION

R1	**IRON (Ferene)**		Reductant
Citric acid	150	mmol/L	
Ascorbic acid	30	mmol/L	
Thiourea	27	mmol/L	
R2	**IRON (Ferene)**		Chromogen
Ferene	600	µmol/L	
R3	**IRON (Ferene)**		Standard

Iron 200 µg/dL (35.8 µmol/L)

I According to 1272/2008 regulation, these reagents are not classified as dangerous.

I SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENTS PREPARATION

Prepare working reagent as follows: R1 (50 volumes) + R2 (1 volume).
Use carefully cleaned material with HCl 0.1 N and well rinsed with distilled water. Give a special care to the quality of water, reagents and/or specimens.
Some automated instrument requires special preparation (see specific procedure).

I STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 2-8°C, and used as described, reagents are stable:

Unopened:

- Until expiry date stated on the label of the kit

Once opened:

- Standard (vial R3): Transfer the requested quantity, recap, and store at 2-8°C
- Reagents are stable at least 12 months without contamination
- Discard reagents if cloudy or if Assay blank > 0.100.

Don't use reagents after expiry date.

SPECIMEN COLLECTION AND HANDLING (6)

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

Serum iron is stable in specimen for:

- 4 days at room temperature.
- 1 week stored 2-8°C.

I LIMITS (3) (5)

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.

I MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Spectrophotometer or Biochemistry Clinical Analyzer

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with

I CALIBRATION (7)

- REF 95015 Multicalibrator traceable to SRM 3126a.
- With manual procedure only: Standard (vial R3)

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

I QUALITY CONTROL

- REF 95010.: Exatrol N Level 1
- REF 95011: Exatrol P Level 2
- 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. Prepare a fresh control serum and repeat the test
2. If control is still out of range, use a new vial of fresh calibrator
3. If control is still out of range, use a new vial of reagent and re-assay

If control is still out of range, please contact BIOLABO technical support or your local Agent.

REFERENCE INTERVAL (2)

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

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

I PERFORMANCES

On Kenza 240TX, 37°C, 620 nm

Linearity: from 27 µg/dL (4.9 µmol/L) to 2198 µg/dL (393 mmol/L)

Detection limit: approximately 2.7 µg/dL (0.49 µmol/L)

Precision:

Within run N = 20	*Level1*	*Level2*	*Level3*
Mean µg/dL	26	132	257
S.D. µg/dL	1.1	2.1	2.7
C.V. %	4.4	1.6	1.1

Between run N = 20	*Level1*	*Level2*	*Level3*
Mean µg/dL	26	134	260
S.D. µg/dL	0.9	2.5	6.2
C.V. %	3.5	1.9	2.4

Analytical Sensitivity for 10 µg/dL: 0.0084 abs

Comparison study with commercially available reagent using human specimens (n=121) from 17 µg/dL to 290 µg/dL:

y = 0.9987x + 0.3847 r =0.9974

Interferences:

Total bilirubin	No interference up to 500 µmol/L
Direct bilirubin	No interference up to 504 µmol/L
Ascorbic acid	No interference up to 2500 mg/dL
Glucose	No interference up to 966 mg/dL
Turbidity	No interference of the turbidity up to 0.278OD
Haemoglobin	Negative interference from 95 µmol/L

Other substances may interfere (see § Interferences)

On the board stability: 2 months

Calibration Stability: 2 months

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations

PROCEDURE

Manual procedure

Let stand reagents and specimens at room temperature.

BLANK-TUBES	Blank	Standard	Assay
Reagent R1	1 mL	1 mL	1 mL
Specimen			200 µL
Standard		200 µL	
Distilled water	200 µL		
Mix gently. Let stand for at least 3 minutes at room temperature. Record A1 absorbance at 600 nm (580-620) against blank.			

ASSAY-TUBES	Blank	Standard	Assay
Working Reagent	1 mL	1 mL	1 mL
Specimen			200 µL
Standard		200 µL	
Distilled water	200 µL		
Mix gently. Let stand for 5 minutes at room temperature. Record A2 absorbance at 600 nm (580-620) against blank. Colour is stable for 1 hour.			

1- Performances with manual procedure should be validated by user.

2- Above linearity limit dilute specimen with saline solution and re-assay considering dilution factor.

3- Kenza applications and other applications proposal are available on request.

CALCULATION

Manual Procedure:

$$\text{Result} = \frac{(A2 - A1)\ \text{Assay}}{(A2 - A1)\ \text{Standard}} \times \text{Standard concentration}$$

Automatic Biochemistry analyzer:

The analyzer provides directly calculated result.

For more details about calibration and calculation of results, refer to User's manual and specific application.

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) *FERENE: a new spectrophotometric reagent for IRON. Douglas J. HENNESY, Gary R. REID, Frank E.SMITH, and Stephen L. THOMPSON, CAN.J. Chem. (1984) 62, p.721-724*

(5) *A systematic evaluation of bathophenantroline, ferrozine 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) *HENRY RJ, (Ed) Clin. Chem., Principles and technics, (2ème éd.), Harper and Row, (1974) p.682-695.*

(7) *SRM: Standard Reference Material ®*