en

Iron

Ferene

General Information

Characteristics

- Ferene – chromogen, no interference by copper, 25 % increased sensitivity compared to ferrozine.
- Stable at room temperature, without corrosive reagents.
- Without foaming detergents – hence simple and clean pipetting resulting in high precision.
- Automatable due to short reaction time.
- Complete separation of iron from transport protein.
- Without protein removal, thus simple working steps and small sample volume.
- High extinction and good linearity: up to 200 µmol/L (1100 µg/dL) Fe.
- Evaluation by extinction coefficient or by standard.
- Quality control with conventional control sera (also of animal origin).

Principle

In acetate buffer transferrin (TF) bound iron dissociates into ferric ions which are reduced to ferrous ions (Fe^{2+}) in the presence of ascorbic acid. AMAH promotes the reaction. The ferrous ion reacts with the chromogen Ferene to form a blue complex (chelate) with an extinction maximum at 590 nm. The method is specific for iron in serum/plasma and the absorbance is directly proportional to the iron concentration up to 200 µmol/L (1100 µg/dL).

$$n Fe^{3+} \cdot TF\,(Transferin) \xrightarrow[\text{(ascorbic acid)}]{\text{(acetate buffer) AMAH}} n Fe^{2+} + n(2H^+) + TF$$

$$2Fe^{2+} + 3\,Ferene^{2-} \Rightarrow 1\,(2Fe \cdot Ferene_3)^{2-}$$

Reagents

The reagents are originally closed and stored at room temperature (max. +25 °C) stable until the expiration date.

Risks and Safety

Please observe the necessary precautions for use of laboratory reagents and body fluids. Applications should be performed by expert personnel only. Follow the national and laboratory internal guidelines for work safety and infection control. Wear suitable protective clothing and disposable gloves while handling.

It is important to ensure effective protection against infection according to laboratory guidelines.

SDS ID International
www.sds-id.com

For additional safety information please refer to the information on the label and the corresponding Safety Data Sheet (SDS).

Download by QR-Code or link:

www.sds-id.com/100055-9 (R1a = Iron Buffer)
www.sds-id.com/100056-8 (R1b = Reducing Reagent)
www.sds-id.com/100058-6 (R2 = Chromogenic Reagent Ferene)
www.sds-id.com/100059-5 (CAL = Calibrator (Standard))

Main Components

006511-...	Cont.	4.50 mol/L AMAH, 1.55 mol/L acetate, 25 mmol/L thiocarbamide
006512-...	Cont.	Ascorbic acid cryst.
006514-...	Cont.	40 mval/L Ferene (buffered pH = 4,5)
006516-...	Cont.	25.0 µmol/L = 140 µg/dl Fe^{3+} (buffered)

006501-6001	**KIT**	**6× 25 mL Iron Ferene**	
006511-0025	R1a	6× 25 mL	Iron Buffer
006512-0025	R1b	6× 50 mg	Reducing Reagent
006514-0006	R2	1× 6 mL	Ferene (Chromogen)
006516-0010	CAL	1× 10 mL	Calibrator (Standard)
006501-6002	**KIT**	**4 × 100 ml Iron Ferene)**	
006511-0100	R1a	4× 100 mL	Iron Buffer
006512-0025	R1b	4× 200 mg	Reducing Reagent
006514-0016	R2	1× 16 mL	Ferene (Chromogen)
006516-0010	CAL	1× 10 mL	Calibrator (Standard)

Spezimen

Serum, heparin plasma: stable at + 4 °C for 7 days, at room temperature for 4 days.

Do not use hemolytic samples.

Preanalytics

Centrifuge samples immediately. When collecting blood, do not use the first 2 ml for Fe determination, as Fe residues may be aspirated from the disposable cannula. This can lead to increased Fe values!

Only use iron-free disposable material.

Reference Ranges

	[µmol/l]	[µg/dl]
Males:	9.5 ... 29.9	53 ... 167
Females:	8.8 ... 27.0	49 ... 151

Increased iron levels may be caused by hormonal contraceptives. The iron level in serum fluctuates day to day up to 30 %, during the day up to 32 %. In the morning the iron level is higher then in the evening. The iron level decreases with aging.

Diagnostic relevance

Due to the high fluctuations of the iron level in sera from day to day, diagnostic conclusions should be drawn only after repeated testing with similar results. A single analysis has only low diagnostic relevance. A useful diagnostic method to complete the analysis of iron in serum is to test the total and latent (unsaturated) iron-binding capacity (TIBC, UIBC).

Diseases, causing decreased iron level:

- Acute blood loss due to severe external or internal bleeding
- Chronic blood loss (e. g. occult gastrointestinal bleeding)
- Menstrual blood loss
- Hookworm infection (Ankylostoma duodenale)
- Iron absorption disorder (e. g. celiac disease)
- Bacterial infections, inflammations, malign tumors
- Nephrotic syndrome, exudative enteropathy, atransferrinemia
- Malnutrition (Fe-uptake < 10 mg/day), increased need for iron (e. g. due to pregnancy, during growth)

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Diseases, causing increased iron level:

- Necrotic liver parenchyma
- Pancreatic insufficiency
- Thalassaemia major
- Vitamin B6 deficiency
- Plumbism
- Idiopathic hemochromatosis

Performance characteristics

Measurement range

The method is suitable for determination of iron concentrations in the range of 1 ... 180 µmol/L (5.6 ... 1005 µg/dL).

If the iron concentration is higher, the samples 1 + 2 have to be diluted with NaCl 0.9 %. The result is then multiplied by factor 3.

Interferences

No significant interference by bilirubin samples up to concentrations of 60 mg/dL, lipemia (triglycerides) up to 1000 mg/dL, copper up to 200 µg/dL. Hemolysis causes increased results.

Precision

Intra-assay n = 20	Mean [µg/dl]	SD [µg/dl]	CV [%]
Sample 1	18.8	0.12	0.63
Sample 2	30.6	0.21	0.67
Inter-assay n = 20	**Mean [µg/dl]**	**SD [µg/dl]**	**CV [%]**
Sample 1	18.8	0.18	0.97
Sample 2	30.8	0.40	1.28

Correlation

The herein described product was compared with an other commercially available kit for its performance for 50 samples. The following correlation results were obtained: y = 0.991 × +0.156; r = 0.996.

Quality control

To control precision and accuracy it is recommended to use a high-quality control serum.

Notes

Centrifuge samples immediately. When taking the blood sample Do not use the first 2 mL for iron analysis, due to possible iron contamination from the disposable needle. This may cause increased results!

Use iron-free disposables only.

Classifications

Not for human diagnostics.

Support / Infoservice

For methodological and technical support, please contact us by E-Mail at support@bioanalytic.de.

Periodically check for updates of this product information on our website.

Feedback

Information from users can be reported to support@bioanalytic.de.

Suggestions for further developments will be considered.

Waste Management

Please observe your national laws and regulations.

Used and expired solutions must be disposed of in accordance with your local regulations.

Inside the EU, national regulations apply that are based on the current, amended version of Council directive 67/548/EEG on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

Decontaminated packaging can disposed of as household waste or recycled, unless otherwise specified.

Ordering Information

Calibrator (Fe-Standard) and Chromogenic Reagent Ferene may not be sufficient for adaption on automatic analyzers and are also available separately if required.

Literature & Footnotes

Legends for the graphic symbols and tags used follow relevant norms or are available on our internet pages.

[1] L. Thomas, Labor und Diagnose.

Iron

Ferene

Sample / Sample Blank - Technique, Manual, Endpoint

Method

Sample/Sample Blank technique, manual, endpoint. Ferene/ascorbic acid method without protein removal. Product information for measuring of iron in serum and plasma. Evaluation by extinction coefficient or by standard. Automatable due to short reaction time.

Attention!

This additional information is a supplement to the product information.
It is also important to observe the information in the product information!

Preparation

R1 Buffer/Blank
For preparation of reducing buffer R1 dissolve the content of one tube reducing reagent 6512 (R1b) in one bottle of iron buffer 6511 (R1a).
Shelf life: stable at +2 ... +8 °C for 14 days, at +15 ... +25 °C for 5 days

R2 Buffer/Chromogen
Add 1 mL chromogenic reagent 6514 per 25 mL R1 (Reducing buffer) and mix carefully.
Add 4 ml 6514 colour reagent per 100 ml R1 (sample blank reagent) and mix.
Shelf life: see R1.

Procedure

Wavelength: ..... 590 nm, Hg 578 nm
Optical path length: ..... 10 mm
Temperature: ..... +20 ... +37 °C
measuring mode: ..... against sample blank value

Add to reaction tube or cuvette:

		RB	CAL	SB	SA
AQ	Aqua p.a.	100 µl	–	–	–
CAL	Standard	–	100 µl	–	–
SA	Sample	–	–	100 µl	100 µl
R1	Buffer/Blank	–	–	500 µl	–
R2	Buffer/Chromogen	500 µl	500 µl	–	500 µl

Mix and measure after 1 ... 60 minutes sample (E_{SA}) against sample blank (E_{SB}).
The reagent blank value (E_{RB}) must be subtracted from the measured values (determined once per series).

Evaluation/Calculation

$$E_{SA} - E_{SB} - E_{RB} = \Delta E_{SA}$$

1. by extinction coefficient (Hg 578 nm):

µmol/L Fe = $\Delta E_{SA} \times 190$

µg/dL Fe = $\Delta E_{SA} \times 1062$

2. by standard:

µmol/L Fe = $\Delta E_{SA} \times (25 / E_{CAL})$

µg/dL Fe = $\Delta E_{SA} \times (140 / E_{CAL})$

3. Conversion:

µg/dL Fe = µmol/l × 5.59

mg/L [ppm] Fe = µmol/l × 0.0559

Nomenclature

CAL = Calibrator (Standard)
E_{SA} = Extinktion/Absorbance Sample
E_{SB} = Extinktion/Absorbance Sample Blank
E_{RB} = Extinktion/Absorbance Reagent Blank
E_{ST} = Extinktion/Absorbance Standard

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E_1 / E_2 - Technique, Manual, Endpoint

Method

E_1/E_2 technique, manual, endpoint. Ferene/ascorbic acid method without protein removal.
Product information for manual (single) determination of iron in serum and plasma. Evaluation using extinction coefficients or via standard.
Can be automated due to short reaction time.

Attention!

This additional information is a supplement to the product information.
It is also important to observe the information in the product information!

Preparation

R1 Buffer/Reduction
For preparation of reducing buffer R1 dissolve the content of one tube reducing reagent 6512 (R1b) in one bottle of iron buffer 6511 (R1a).
Shelf life: stable at +2 ... +8 °C for 14 days, at +15 ... +25 °C for 5 days

R2 Ferene (Chromogen)
Solution ready to use.

Procedure

Wavelength: ..... 590 nm, Hg 578 nm
Optical path length: ..... 10 mm
Temperature: ..... +20 ... +37 °C
Measuring mode: ..... E_1/E_2

Add to a cuvette:

SA	**Sample**	**100 µl**
R1	**Buffer/Reduction**	**500 µl**

Mix and set photometer to zero ($E_1 = 0$). Then add:

R2	**Ferene (Chromogen)**	**20 µl**

Mix and measure after 1 ... 60 minutes (E_2).

The reagent blank value (E_{RB}) must be subtracted from the measured values (determined once per series). For this purpose, Aqua p.a. is used as a sample. For calculation via standard (CAL), this is also treated as a sample.

Evaluation/Calculation

$E_2 - E_{RB} = \Delta E_{SA}$

1. by extinction coefficient (Hg 578 nm):

µmol/L Fe $= \Delta E_{SA} \times 190$

µg/dL Fe $= \Delta E_{SA} \times 1062$

2. by standard:

µmol/L Fe $= \Delta E_{SA} \times (25 / E_{ST})$

µg/dL Fe $= \Delta E_{SA} \times (140 / E_{ST})$

3. Conversion:

µg/dL Fe = µmol/L × 5.59

mg/L [ppm] Fe = µmol/L × 0.0559

Nomenclature

CAL = Calibrator (Standard)
E_{SA} = Extinktion/Absorbance Sample
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