Iron

CYPRESS DIAGNOSTICS

CE

IVD

Ferrozine. Colorimetric

Store at 2 - *8* °C

Configuration

REF	HB012
VOL	4 x 50 mL
Reagent 1	4 x 50 mL
Reagent 2	4 x 500 mg
Reagent 3	2 x 10 mL
Standard	1 x 10 mL

Intended use

The Cypress Diagnostics kit Iron is an in vitro diagnostic medical device intended to be used for the quantitative measurement of iron in human serum or heparinized plasma. The device is not automated. The measurement of iron is intended to be used to aid to identify iron-deficiency (anemia), hemochromatosis or liver disorders, in patient risk population. This kit is intended to be used by healthcare professionals in a laboratory-based testing environment.
For *in vitro* diagnostic use only. For professional use only.

Clinical significance

The Iron content of the human body may be divided into three classes: iron in storage, iron in use and iron in transport. Increases in serum iron levels may indicate increased erythrocyte destruction, decreased erythrocyte formation, increased absorption or defects in storage capacities. Decreases in serum iron levels may indicate Iron deficiency or inability to retrieve storage iron. Iron binding capacity is usually increased in iron deficiency anemia and decreased in hemochromatosis, malignancies, rheumatic fever, Hodgkin's diseases and collagen vascular disease.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Principle

In serum iron is bound to transferrin. In weakly acidic the Iron dissociates from this complex and the serum proteins remains in solution. After reduction with ascorbic, the iron is converted to a complex by specific color reagent of ferrozine.

$$\text{Transferrin } (Fe^{3+})_2 + e^- \xrightarrow{Ascorbic\ acid} 2\ Fe^{2+} + \text{Transferrin}$$

$$Fe^{2+} \xrightarrow{Ferrozine} \text{Colored complex}$$

The intensity of the color formed is proportional to the iron concentration.

Reagent composition

Reagent 1	**Buffer** Acetate pH 4,9 (100 mmol/L)
Reagent 2	**Reductant** Ascorbic acid (99,7%)
Reagent 3	**Color** Ferrozine (40 mmol/L)
Standard	Iron aqueous (see value on label)

Precautions

- All body fluid samples should be considered potentially infectious materials and the appropriate precautions should be taken. Wear personal protective equipment such as gloves, safety glasses, lab coats or aprons when working with possible biohazard contaminants.
- Use Good Laboratory Practices (GLP) when handling this product.
- Please refer to the MSDS, available on our website, for further information.

Preparation

R3 is ready to use.
Add the contents of one tube R2 reductant to the contents of one bottle R1 buffer. Cap and mix gently to dissolve content. This working reagent is stable for 3 months at 2-8 °C or 1 month at room temperature (15-25 °C).

Storage, stability and disposal

All the components of the kit are stable up to the date of expiration as specified on the label, when stored tightly closed, protected from light and contaminations prevented during their use. Storage temperature for this kit is 2 - 8 °C.
Handle standard very carefully to prevent contamination. The reagent should be a clear solution. If turbidity or precipitation has occurred or if the reagent blank absorbance at 562 nm ≥ 0,020, the reagent should be discarded.
Do not use the product if deterioration or contamination is suspected or beyond the expiration date or open container stability period. Dispose unused or deteriorated product and waste in compliance with local regulations.

Additional material required but not provided

- Spectrophotometer or colorimeter. Minimum analyzer specifications:
  Measuring at 562 nm (530-590)
  Linear measuring range: 0 - 2 AU
- Cuvettes, matching the analyzer used (1,0 cm light path)
- General laboratory equipment[Note 4]

Samples

Sample type: human serum or heparinized plasma Hemolysis interferes with the test. Separate from the cells as rapidly as possible. The iron is stable up to 7 days stored at 2 - 8 °C.

Procedure

Make sure the reagents and samples are at room temperature.
1. Wavelength 562 nm (530-590); Temperature 37 °C/15-25 °C; Cuvette (1 cm light path).
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:[Note 6]

For Reagent Blank	200 µL Distilled water + 1 mL Working reagent + 1 drop R3
For Standard Blank	200 µL Standard + 1 mL Working reagent
For Sample Blank	200 µL Sample + 1 mL Working reagent
For Standard[Note 3]	200 µL Standard + 1 mL Working reagent + 1 drop R3
For Sample	200 µL Sample + 1 mL Working reagent + 1 drop R3
Mix and wait 5 minutes at 37 °C or 10 minutes at room temperature (15-25 °C). Read absorbance (A) of standard and sample against standard/sample blank. The color is stable for at least 30 minutes.	

Calculation

$$\text{iron (µg/dL)} = \frac{A_{Sample} - A_{Sample\ blank}}{A_{Standard} - A_{Standard\ blank}} \times \text{standard conc. (µg/dL)}$$

Conversion Factor: µmol/L = 0,179 x µg/dL

Quality control

Control sera are recommended for the qualification of the reagents and to monitor the performance of assay procedures. Next to legal requirements, we recommend to perform Quality Control on a daily basis and after each calibration.
Use Biochemistry Normal and Pathological Controls Specific (HBC01-S, HBC02-S). If other controls (not manufactured by Cypress) are used, they have to be validated by the user as they can vary. Prepare and measure these controls the same as samples. Measure at least one replicate per control.
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems. Do not continue testing as the results will be invalid. Each laboratory should establish its own QC scheme and corrective actions if controls do not meet the acceptable tolerances.

Reference values[Note 5]

Men	65 - 175 µg/dL (11,6 - 31,3 µmol/L)
Women	40 - 150 µg/dL (7,2 - 26,9 µmol/L)

These values are for orientation purpose. Each laboratory should establish its own reference range.

Performance characteristics

Measuring range: from 0,85 µg/dL (detection limit) to 1000 µg/dL (linearity limit). If the obtained results are greater than 1000 µg/dL, dilute the sample 1:2 with saline solution, repeat the determination, and multiply the result by factor 2.

Precision:

	intra-assay (n=20)		inter-assay (n=20)	
Mean (µg/dL)	113	250	111	249
SD	0,89	0,72	3,51	6,29
CV (%)	0,79	0,29	3,17	2,52

Sensitivity: 1 µg/dL = 0,00104 AU

Accuracy: Results obtained using CYPRESS DIAGNOSTICS reagents did not show systematic differences when compared with other commercial reagents.
The results of the performance characteristics depend on the analyzer used.

Interferences

Hemolyzed samples should be rejected. A list of drugs and other interfering substances with Iron determination has been reported[3,4].

Notes

1. For best use of this kit on a Cypress Diagnostics analyzer, we kindly advise to follow the application sheets of the respective analyzer. Please log in to our website (www.diagnostics.be) as a registered user to download the latest application sheets, which are located under the section of the corresponding analyzer. Compatible Cypress analyzers: CYANSmart, CYANStart, CYANExpert 130, CYANVision

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2. In case other instruments (not manufactured by Cypress Diagnostics) are used, the laboratory is responsible to validate the reagents in this kit on those analyzers before testing patient samples.
3. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In this case, it is recommended to use a serum based Biochemistry Calibrator Specific (HBC03-S).
4. It is recommended to use disposable material. If glassware is used, the material should be soaking for 6 hours in diluted HCl (20% v/v) and then thoroughly rinsed with distilled water and dried before use.
5. The reference values are strongly method dependent.
6. Use clean disposable pipette tips for its dispensation.

Bibliography

1. Perotta G. Iron and iron-binding capacity. Kaplan A et al. Clin Chem The C.V. Mosby CO. St Louis. Toronto. Princeton 1984; 1063-1065
2. Itano M.M.D. Cap Serum Iron Survey 1978, 70: 516-522
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press 1995
4. Young DS. Effects of diseases on Clinical Lab. Tests, 4th ed AACC 2001
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999
6. Tietz N W et al. Clinical Guide to Laboratory tests, 3rd ed AACC 1995.

Notice: Any serious incident that has occurred in relation to the device shall be reported to Cypress Diagnostics and the competent authority of the Member State in which the user and/or patient is established.

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