

Manual Procedure

Cat. No. 12480 R 1 x 50 ml
For 50 tests

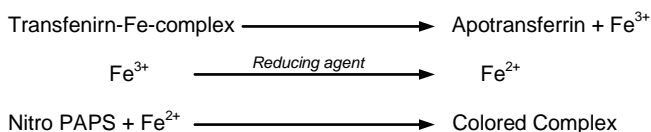
Cat. No. 12481 R 2 x 50 ml
For 100 tests

Iron (Nitro - PAPS)

Colorimetric Method

Liquid Reagent

Test principle



Iron ions are dissociated from its carrier protein (transferrin) in an acid medium and are reduced to the ferrous form by a reducing factor. The ferrous ions react with the chromogen Nitro-PAPS to form a colored complex highly specific. The absorbance of the color is directly proportional to the iron concentration.

Concentrations in the test

Reagent R		
Guanidine hydrochloride pH = 4.8	2.2	mol/L
Nitro PAPS	26	μmol/L
Thio urea	60.0	mmol/L
Reducing agent		
Detergent		
Standard : The concentration as indicated on vial.		

Stability of reagent

Reagent R: liquid, ready to use.

The reagent is stable up to expiry date given on the label when stored at +2 → +8 °C.

Note: Don't use if the reagent is turbid.

Specimen collection and handling

- Fresh, non-hemolyzed serum is the specimen of choice.
- Heparinized plasma could only be used, other anticoagulants should not be used.
- Serum or plasma should be separated as soon as possible.
- Serum iron is reported to be stable for 4 days at 20 - 25°C, and 7 days at 2 - 8 °C.

Calibrator / Standard

MediCal U Cat. No. 15011
Iron STD. Cat. No. 16131

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 578 nm
Spectrophotometer	582 nm
Cuvette	1 cm light path
Temperature	37 °C / 20 - 25°C
Measurement	against reagent blank
Reaction	end point

Assay

	Blank	Calibrator / Standard	Sample
Double dist. water	100 μl	--	--
Calibrator / Standard	--	100 μl	--
Sample	--	--	100 μl
Reagent	1000 μl	1000 μl	1000 μl

Mix, incubate for 5 min. at 37°C or 10 min. at 20 - 25°C. Read the absorbance (A). The final color is stable for 1 hour.

Calculation

$$\text{Conc. Iron } (\mu\text{g/dl}) = \frac{A_{\text{Sample}}}{A_{\text{Cal./STD}}} \times \text{Conc. Cal./STD } (\mu\text{g/dl})$$

$$\mu\text{mol/L} \xleftrightarrow[0.179 \times]{\times 5.58} \mu\text{g/dl}$$

Linearity

Up to 400 μg/dl

If the result exceeds 400 μg/dl, repeat the test using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Interferences

- Contaminated glassware is the greatest source of error. Disposable material is recommended to avoid iron contamination.
- Triglycerides up to 400 mg/dl have been found to significantly influence this assay.
- To make glass material iron-free rinse with diluted (1+2) hydrochloric or nitric acid followed by several rinsings with iron free deionized or distilled water.
- Certain drugs and other substances are known to influence circulating iron levels.

Precautions

- The reagent is toxic, don't pipette by mouth, and avoid all contacts.
- Use only disposable plastic containers or zinc free tubes and cuvettes. Avoid any contamination by the use of clean laboratory material.

Reference range

Serum

New born	36 - 184	μg/dl
≤ 6 mth.	36 - 156	μg/dl
≥ 7 mth.	43 - 184	μg/dl
Adults	women	37 - 145
	men	59 - 158
		μg/dl

Urine

Urine /24 hr.	< 98	μg/ 24 hr.
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References

- Makino et al :Clinica Chemica Acta, 171 (1988), 19 – 28.
- Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.