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IRON FERROZINE

STABLE LIQUID REAGENT

CE

Cat. No.::	45663	45661,45661S
	10x25 ml	125 ml
	(10x20ml+10x5ml)	(1x100ml+1x25ml)

Reagent kit for determination of iron concentration in serum. Colorimetric method.

Principle
At pH=4.8 and in presence of ascorbic acid, trivalent iron [Fe(III)] dissociated from the transferrin becomes reduced to divalent iron [Fe(II)] which forms a red complex with ferrozine. The absorbance read at 570 nm is proportional to the iron concentration of sample.

Reference values
Serum total iron: **Female:** **8.8-27 µmol/l (49,2-150,9 µg/dl)**
Male: **9.0-30 µmol/l (50,3-167,7 µg/dl)**
It is recommended that each laboratory should assign its own normal range. The reference value is influenced by the eating
habits, sex, age, physical state, menstrual period, pregnancy, effects of environment.

Reagents
1.Reagent (R1)

Acetate buffer, pH=4.80	100 mmol/l
Guanidine	6 mmol/l
Thiourea	52.5 mmol/l
Reducing agent	

2.Reagent (R2)

Ferrozine	40 mmol/l

3. Iron standard
Ready for use.For details please check the insert.
Available only in Cat. No.: 45661S

Safety instructions:
Reagent 1:
X, Harmful
R22 Harmful if swallowed.
R36/38 Irritating to eyes and skin.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

Sample
Serum free of haemolysis.

PROCEDURE

Preparation and stability
- **Working reagent:** mix 4 volume of R1 with 1 volume of R2. Stable for 4 weeks at 2-8°C
- **Two reagent:** the reagents are ready for use

Assay conditions:

Wavelength:	570 nm
Secondary wavelength:	600 nm
Temperature:	37 °C
Cuvette:	1 cm pathway
Method:	endpoint (increasing)
Reading against:	reagent blank

Method:

Two reagent procedure:

	blank	standard	sample
R1	800 µl	800 µl	800 µl
Distilled water	100 µl		
Standard		100 µl	
Sample			100 µl

Mix and incubate for 3 minutes

R2	200 µl	200 µl	200 µl

Mix and read the absorbance (A) after a 5-minute incubation against reagent or sample blank.

Working reagent method:

	blank	standard	sample
Working reagent	1000 µl	1000 µl	1000 µl
Distilled water	100 µl		
Standard		100 µl	
Sample			100 µl

Mix and read Absorbance (A) after 5 minutes incubation against reagent blank

Calibration: (37°C, colorimetric method)
S1: Distilled water
S2: Iron standard Cat. No.: 51111 or
Diagnosticum Dunacal
Randox Calibration Serum Level I or
Randox Calibration Serum Level II

Calibration frequency
Two point calibration is recommended
- after reagent lot change,
- as required following quality control procedures.

Calculation

$$\frac{A_{sample}}{A_{standard}} xC_{standard} = C_{sample}$$

A = absorbance,
C= concentration

Quality control
A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Olympus 600 analyzer (37°C).

Linearity
The test is linear up to 179 µmol/l (1000µg/dl) iron concentration.

Sensitivity
It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs is equivalent to 0.20 µmol/l (1,11 µg/dl) iron concentration at 578 nm.

Precision

	Reproducibility		
	Average conc. (µmol/l)	SD	CV%
Sample I.	20.9	0.214	1.02
Sample II.	36.1	0.753	2.09

	Repeatability		
	Average conc. (µmol/l)	SD	CV%
Sample I.	10.9	0.146	1.35
Sample II.	27.9	0.334	1.20

Correlation
Comparative studies were done to compare our reagent with another commercial Iron ferrozine assay.
The results from these studies are detailed below.
Correlation coefficient: r=0.9971
Linear regression: y (µmol/l)= 0.954x+1.070
(x=other commercial reagent y= own reagent).

Specificity

Hemoglobin 1.6 µmol/l (10 mg/dl), bilirubin 736 µmol/l (43 mg/dl), lipid 800 mg/dl, glucose 55.5mmol/l (1000 mg/dl) and ascorbic acid 2.84 mmol/l (50 mg/dl) don't interfere with the assay up to the given levels.

Note
Haemolysis interferes with the test. Chelating compounds can not be used as anticoagulants. Thiourea prevents disturbing effects of copper ions.
Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For *in vitro* diagnostic use only.

The following symbols are used on labels

IVD For in vitro diagnostic use

Use by (last day of the month)

Temperature limitation

LOT Batch Code

REF Code

Bibliography
Williams et al.: Clin. Chem., 23, 237 (1977)
Stoockey L.: Anal. Chem., 42,779 (1970)
Persijn et al.: Clin. Chem. Acta, 35, 91 (1971