

Cat. No.:	615635	615633
	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

### Sample

Serum free of haemolysis.

### PROCEDURE

#### Assay conditions:

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

The reagents are ready to use

### Method:

#### Two reagent procedure:

	blank	standard	sample
<b>R1</b>	800 µl	800 µl	800 µl
<b>Distilled water</b>	100 µl		
<b>Standard</b>		100 µl	
<b>Sample</b>			100 µl

Mix and incubate for 3 minutes

<b>R2</b>	200 µl	200 µl	200 µl
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Mix and read the absorbance (A) after a 5-minute incubation against reagent blank.

#### Working reagent method:

	blank	standard	sample
<b>Working reagent</b>	1000 µl	1000 µl	1000 µl
<b>Distilled water</b>	100 µl		
<b>Standard</b>		100 µl	
<b>Sample</b>			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.: 151111 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

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}}$$

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%
<b>Sample I.</b>	20.9	0.214	1.02
<b>Sample II.</b>	36.1	0.753	2.09

	Repeatability		
	Average conc. (µmol/l)	SD	CV%
<b>Sample I.</b>	10.9	0.146	1.35
<b>Sample II.</b>	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

Xn  
Harmful

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)

Stookey L.: Anal. Chem., 42,779 (1970)

Persijn et al.: Clin. Chem. Acta, 35, 91 (1971)