IRON
(Ferrozine Method)

Diagnostic reagent for determination of Iron concentration.

Liquid. Dual Reagents. Store at 2°C - 8°C. For in Vitro Diagnostic Use. Do not freeze

Ref No	Pack	Ref No	Pack	Ref No	Pack	Ref No	Pack
A2240	5 x 100 mL	R2240	2885 Tests	L2241	2000 Tests	N2240	800 Tests
A2241	5 x 50 mL	R2241	962 Tests	S2240	3077 Tests	N2241	400 Tests
T2240	3882 Tests	M2240	2512 Tests	S2241	1477 Tests	K2240	5455 Tests
T2241	2059 Tests	M2241	1675 Tests	BY2240	6136 Tests	K2241	3636 Tests
DM2240	630 Tests	L2240	3750 Tests	BY2241	4091 Tests		

INTENDED USE

The test is applied for the quantitative determination of iron concentration in human serum.

TEST PRINCIPLE

Serum Iron: Transferrin-bound iron is realized at an acid pH an reduced from ferric to ferrous ions. These ions react with ferrozine to from a violet colored complex which is measured spectrophotometrically at 560 nm. The absorbance measured at this wavelength is proportional to serum ion concentration.

TEST PARAMETERS

Method	: Colorimetric, Endpoint, Increasing Reaction
Wavelength	: 578 nm (560-580) 2.WL:700-750nm
Temperature	: 37 °C
Sample	: Serum
Linearity	: 10 µg/dL - 500 µg/dL

REAGENT COMPOSITION

Iron Buffer (Reagent 1):

Acetate buffer	pH 4.5
Hydroxylamine hydrochloride	≤ 220Mm

Reagent 2:

Ferrozine	≤ 15 g/L
Buffer	
Antibacteriel	

Iron Standard:
Ferrous chloride
Hydroxylamine hydrochloride

REAGENT PREPARATION

Reagents are ready to use.

Working reagents are stable at 2-8ºC in case of closed vials and avoiding contamination after preparation.

For manual working procedures; if working reagent will be used; shake the Reagent 2 vial gently before pouring its contents into the Reagent 1 bottle. It is advisable to wash the Reagent 2 vial with a small volume of the prepared mixture in order to completely rinse the vial and avoid any losses.

REAGENT STABILITY AND STORAGE

Once opened vials are stable minimum 30 days at 2-8°C at optimum conditions. On board stability is strongly related to auto analyzers cooling specification and carry-over values.

SAMPLE

Serum is collected by standard procedures.
Sample in serum is stable for 7 days at 20 - 25°C, 3 weeks at 2-8ºC and minimum 1 year at -20°C.

TEST PROCEDURE

Sample Start
There have many ready application procedures dedicated to different kind of photometers and ready manual working process can be supplied on request.

There have many ready application procedures dedicated to different kind of biochemistry auto analyzers can be supplied on request.

Substrate Start
There have many ready application procedures dedicated to different kind of biochemistry auto analyzers can be supplied on request.

CALCULATION

$$\frac{A \text{ Sample}}{A \text{ Standard}} \times \text{conc. of standard}$$

= Iron in sample (µg/dL)

Unit Conversion

µg/dL x 0.1791 = µmol/L

REFERENCE INTERVAL (NORMAL VALUES) (Based on CLSI C28-P Document)*

Total Iron: Women: 37 - 145 µg/dL
                  Men   : 60 – 150 µg/dL

*It is recommended that each laboratory establish its own reference range.

QUALITY CONTROL AND CALIBRATION

Commercially available control material with established values determined by this method may be used.
We recommend:

"ARCON N", Assayed Control Serum Normal
Cat.No. A3910

"ARCON P", Assayed Control Serum Abnormal
Cat.No. A3920

The assay requires the use of an Iron Standard or an Iron Calibrator. We recommend:
ARCHEM Standard
Cat.No. IR2240DS

*Any commercially available Standard or Calibrator suitable for this method may be used.

*Calibration Stability: It is strongly depend of application to auto analyzers and auto analyzers specification. Calibration stability is 25 days.

*Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

Quality control is recommended every morning. Calibration is not recommended if QC control values are acceptable. Reagent should be calibrated after lot changes.

PERFORMANCE CHARACTERISTICS

Low linearity: 10 µg/dL.

High Linearity: The method is linear up to 500 µg/dL.

If the limit value is exceeded, it is suggested to dilute sample 1+9 with distilled water and to repeat the test, multiplying the result by 10.

Linearity may considerably vary depending on the instrument used.

Precision Studies (Based on CLSI EP5 Doc.):

Repeatability (within run) (Intra-assay)

Mean conc.	SD	CV	n
111.70 µg/dL	2.31	2.10%	10
172.10 µg/dL	2.28	1.30%	10

Reproducibility (run to run) (Inter-assay)

Mean conc.	SD	CV	n
111.79 µg/dL	2.66	2.40%	20
170.58 µg/dL	2.53	1.50%	20

Sensitivity (LOD) (Based on CLSI EP17 document): The limit of detection is 12 µg/dL.

Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

Interferences: No interference was observed by the presence of:

Hemoglobin	≤ 80 mg/dL
Bilirubin	≤ 50 mg/dL
Lipids	≤ 100 mg/dL

Methods comparison: A comparison between Archem and a commercially available product gave the following results:
Iron Archem = x
Iron competitor = y
n = 95
y = 1.08x - 8.71 µg/dl r^2 = 0.97

These performance characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure is used.

NOTES

1. For in vitro diagnostic use only. Do not pipette by mouth. Avoid contact with skin and mucous membranes.
2. All the calibrators, controls and some reagents must be considered as human & animal sample, so potentially infectious; all the protection actions must be applied to avoid any potential biological risk.
3. Material safety data sheet will be supplied on request.
4. Exercise the normal precautions required for handling laboratory reagents.
5. After measurements are taken, reagent bottles should cap and kept at 2-8°C. Caps of the reagents bottles cannot be used between two different kind of reagent and between R1&R2.
6. Reagents with different lot numbers should not be interchanged or mixed.
7. The linearity limit depends on the sample to reagent ratio.

PRECAUTIONS AND WASTE DISPOSAL

This product is made to be used in professional laboratories and by professional operators. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

R36/38 : Irritating to eyes and skin.
S20/21 : When using, do not eat, drink or smoke.
S26 : In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 : After contact with skin wash immediately with plenty of water.
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
S45 : In case of accident or if you feel unwell, seek medical advice immediately.
S56 : Dispose of this material and its container at hazardous or special waste collection point.
S57 : Use appropriate container to avoid environmental contamination.
S61 : Avoid release in environment. Refer to special instructions/safety data sheets.
Please consult local regulations for a correct waste disposal.

ABBREVIATIONS

CLSI : Clinical and Laboratory Standards Institute
CV% : Coefficient of Variation Percentage
EP : Evaluation Protocols
GLP : Good Laboratory Practice
IU : International Unit
mA : miliabsorbance
mL : mililiter
NCCLS : National Committee for Clinical Laboratory Standards
QC : Quality Control

REFERENCES

1. Tietz, NW, Textbook of Clinical Chemistry, W.B. Saunders Co, Philadelphia, PA 1986, pp 52-53 (techniques and procedures to minimize laboratory infections) and pp 478-497 (specimen collection and storage recommendations).
2. Tietz NW. Clinical Guide to Laboratory Tests. 3rd ed. Philadelphia, PA: WB
3. Young DS. Effects of Drugs on Clinical Laboratory Tests. 3rd ed. Washington: Press; 1990.
4. Artiss JD, Vinogradov S, Zak B. Spectrophotometric study of several sensitive reagents for serum iron. Clin Biochem. 1981;14:311-315.
5. Siedel J. Improved Ferrozine based reagent for the determination of serum iron (transferrin iron) without deproteinization. Clin Chem. 1984;30:975.
6. Clinical and Laboratory Standards Institute (formerly NCCLS). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2004. NCCLS Document EP05-A.
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SYMBOLS

Symbol	Description
IVD	Only for invitro diagnostic use
LOT	Lot of manufacturing
R1	Reagent 1
R2	Reagent 2
CONC	Concentration
INGRED	Reagent Ingredients
REF	Reference Number (Catalog No)
SN	Serial Number
	Expiration date
	Storage temperature interval
	Read the directions
	Biological risk

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