IRON FERROZINE
Multi-Purpose (MPR) Liquid Reagent

Glenbio
Diagnostics & Research Laboratory Solutions

KIT SPECIFICATIONS:

Cat. No.	Quantity	Reagent	Storage
GL321F	10 x 15 ml	IRON – 1	2 - 8°C
	10 x 15 ml	IRON - 1a	
	2 x 15 ml	IRON – 2	
GL331F	5 x 50 ml	IRON – 1	2 - 8°C
	5 x 50 ml	IRON - 1a	
	1 x 50 ml	IRON – 2	

INTENDED USE:

In Vitro Diagnostic reagent pack for the determination of Iron in serum and plasma on automated and semi-automated analysers.

SUMMARY AND EXPLANATION: [1]

The average diet of Iron per day is 10 to 15 mg. Most of this is in the form of haemoglobin and myoglobin from meat. Normally 1 mg of Iron is absorbed each day. Absorption occurs principally in the duodenum. Haem is absorbed directly; inorganic iron is absorbed in the ferric state. Iron measurements are used in the diagnosis and treatment of diseases such as iron deficiency anaemia, haemochromatosis and chronic renal disease.

PRINCIPLE OF THE TEST: [2, 3]

Sample is added to a buffer containing ascorbic acid to reduce the ferric to the ferrous iron. Ferrous iron reacts with ferrozine to form a strongly purple coloured complex. The absorbance of this complex is directly proportional to the concentration of iron in the sample.

Transferrin Fe Complex $\xrightarrow{pH<2}$ Apotransferrin + Fe^{3+}

$Fe^{3+} \xrightarrow{Ascorbate} Fe^{2+}$

Fe^{2+} + Ferrozine $\longrightarrow$ Coloured Complex

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only - For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

Components Colour and Appearance:
Reagent 1: Clear colourless liquid.
Reagent 2: Clear, pale yellow liquid. .
Reagent 1a: White powder.
Any significant changes from the above could indicate that the assay might be compromised. Refer to Laboratory's QC program for actions to be taken. In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.

Safety precautions:
CAUTION: Take all necessary precautions required when handling laboratory reagents. Material Safety Data Sheet is available upon request.

Label Elements:

WARNING

H302 – Harmful if swallowed.
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary Statements:
P264 – Was skin thoroughly after handling.
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P301 + P312 – IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P330 – Rinse mouth.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P362 – Take off contaminated clothing and wash before reuse.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P501 – Dispose of contents/container according to local guidelines.

Handling precautions:
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.

INSTRUMENTS:

Instrument applications are available upon request.

COMPONENT COMPOSITION:

Component	Ingredients	Concentration in Tests
Reagent 1	Guanidine	837 mmol/l
	Sodium Acetate	99.9 mmol/l
	DETERGENT	---
Reagent 1a	L-Ascorbic Acid	1 g / 100 ml
Reagent 2	Ferrozine	1.52 mmol/l
Standard	Iron	18 µmol/l (100 µg/dl)

REAGENT PREPARATION AND STABILITY:

Before use, mix reagent by gently inverting each bottle.
Working Reagent 1: Dissolve one vial of Reagent R1a into one bottle of Reagent 1.
Stability: Working reagent is stable for 1 *month at 2-8 ℃*.
Reagent 2 is ready for use.
If stored and handled properly, components are stable until expiry date stated on the label.

TYPE OF SPECIMEN: [1]

Use serum or heparin plasma, as specimen. *Do Not Use* Oxalate and EDTA.
It is recommended to follow NCCLS procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sample container, with proper specimen identification. Serum/plasma should be separated from cells within 1 hour after collection.
Stability: up to 3 weeks at 4°C[4].

TEST PROCEDURE:

Materials required but not supplied:

Description	Catalog. No.	Description	Catalog. No.
General Chemistry Control Level 1	GL922	Photometer	N/A
General Chemistry Control Level 2	GL932	General Laboratory Equipment	N/A

Assay procedure:
Wavelength: λ: 560 nm
Temperature: 25°C, 30°C or 37°C
Optical path: 1 cm light path.

	Blank	Calibrator	Sample
Reagent 1	1000 µl	1000 µl	1000 µl
Sample	----	----	100 µl
Calibrator	----	100 µl	----
Gently mix and Incubate at 37°C Measure the Optical Density (OD1) after 5 minutes.			
Reagent 2	200 µl	200 µl	200 µl
Gently mix and Incubate at 37°C Measure the Optical Density (OD2) after 5 minutes.			

Calibration:
Use recommended Calibrator to calibrate the assay:
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Controls are out of range.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At intervals established by the Laboratory QC Programme.

CALCULATION:

$$\text{Concentration of Iron} = \frac{\text{Sample}_{OD2} - \text{Sample}_{OD1}}{\text{Calibrator}_{OD2} - \text{Calibrator}_{OD1}} \times \text{Concentration of Calibrator}$$

*Photometer must be blanked with the reagent blank. (Conversion Factor: Qty in µg/dl x 0.179 = Qty in µmol/l)

EXPECTED VALUES: [1]

	µmol/l	µg/dl
Men	11.6-31.3	64.8 – 175
Women	9.0-30.4	50.3-170

Each laboratory should establish its own reference range. Iron should always be reviewed with the patient's medical examination and history.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity:
This assay is linear up to 70µmol/l (395 µg/dl).
For samples with a higher concentration, dilute 1:1 with 0.9% NaCl (9g/l) and re-assay. Multiply result by 2.

Interfering substances:
Results of study are as follows:

Bilirubin (mixed isomers):	Less than 10% interference up to 600µmol/l Bilirubin.
Haemolysis:	Less than 10% interference up to 2.5 g/l Haemoglobin.
Lipemia:	Less than 10% interference up to 5 g/l Intralipid.

Sensitivity:
The Lowest Detectable Level was estimated at 0.4 µmol/l

Precision:

Within Run N = 20	Mean (µmol/l)	SD	% CV	Between Run N = 20	Mean (µmol/l)	SD	% CV
Level 1	20.9	0.72	3.45	Level 1	21.0	0.63	3.01
Level 2	37.1	0.91	2.46	Level 2	35.6	1.03	2.89

Method Comparison:
Using 50 samples, a comparison, between this Iron Ferrozine test (y) and another commercially available test (x), gave the following results:

y = 0.912x + 2.617	r = 0.990	Sample range: 5.5 to 59.2 µmol/l

BIBLIOGRAPHY:

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2. Stookey LL. Anal Chem 1970; 42: 779.
3. Ruuta R. Clin Chem Acta 1975; 61: 229-232.
4. Guder WG, Narayanan S, Wisser H, Zawta B. The Quality of Diagnostic Samples. Brochure in: Samples: From the Patient to the Laboratory, 2nd edition. Darmstadt: GIT Verlag, 2001.

SYMBOLS:

The following symbols are used in the labelling of Glenbio Ltd systems:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	STD	Aqueous Standard
CE	CE Mark - Device comply with the Directives 98/79/EC		
	Storage temperature	→	Reconstitute with
	Expiry Date (Last day of the month)		Manufactured By
	Biological risk		Consult Instruction for Use

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