PRODUCT INFORMATION

Iron Assay (Ferrozine Chromogenic Method)

Cat. No. KT-757

INTENDED USE

The Iron Assay (Ferrozine Chromogenic Method) is a direct colorimetric assay for the quantitative determination of iron in biological samples that does not require sample deproteinization. For research use only. Not for use in diagnostic procedures in the U.S.

PRINCIPLE

Iron binding to a transport protein such as transferrin is dissociated by a weak acidic buffer and a denaturating agent. Dissociated iron is then reduced and forms a chelate with the Ferrozine chromogen. The intensity of this colored complex is proportional to the iron concentration in the sample and is measured at 560 nm.

COMPONENTS (~200 TESTS)

1. Buffer, 40 mL x 1
2. Chromogen (Ferrozine), 1.6 mL x 1
4. Iron Calibrator (200 µg/dL), 8.0 mL x 1

Store all kit components at 4ºC.

PRECAUTIONS

1. Fluctuating incubation temperature may result in variable results.

2. Use disposable test tubes and glassware washed with 1M HNO_3 or 1M HCl solution and distilled water.

3. Sample and reagent pipetting accuracy may affect assay performance. Please note that samples, calibrator, and reagents must be dispensed accurately at the µL level.

4. The temperature of the reaction may affect the O.D. reading. Please extend or shorten the chromogen reaction time depending on the ambient room temperature if necessary.

5. For cell lysates or the tissue extraction samples, a high concentration of proteins or lipids may affect the assay result. For best results, remove proteins or lipids by ultrafiltration or centrifugation.

6. Heme-containing iron species cannot be measured by this assay kit.

SAMPLE PREPARATION

1. Serum or plasma

Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation. EDTA-plasma samples cannot be used as EDTA interferes with this assay.

2. Tissue extracts, cell lysates, and other samples such as urine or other biological fluids:

If the sample is turbid, centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use for the assay.

If necessary, add small amounts of 6M HCl to the sample and adjust pH to 2.0 - 3.0. For example, add ~5-10 µL of 6M HCl per 1 mL of sample.

3. Tissue samples

Add 3% TCA solution, vortex 1 min. and incubate at 4 - 8℃ for 30 min. Centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use for the assay.

Sample pH should be between pH 2.0 to pH 8.0.

REAGENT PREPARATION

Reagents are ready to use.

Bring all reagents to room temperature before use.

ASSAY PROTOCOL (Microplate and Microplate Reader)
(Total reaction volume = 248 µL)

1. Add 200 µL of Buffer to each well.

2. Add 40 µL of Blank (purified water), Iron Calibrator, or Sample to each well. Mix carefully using a pipette and avoid foaming. If a plate mixer is used for mixing, there is a risk of obtaining poor reproducibility. Incubate at room temperature for 5 minutes.

3. Read the absorbance at 560 nm (main) to give OD1 values.

4. Add 8 µL of Chromogen to each well, mix, and incubate at room temperature for 5 minutes. Mix carefully using a pipette and avoid foaming. If a plate mixer is used for mixing, there is a risk of obtaining poor reproducibility

5. Read the OD absorbance at 560 nm (main) of each reaction to give OD2 values.

Assay Protocol				
Step	(µL)	Blank	Calibrator	Sample
1	Buffer	200	200	200
2	Purified water	40	-	-
	Iron Calibrator	-	40	-
	Sample	-	-	40
3	Mix and incubate for 5 minutes at room temp. Read the absorbance (OD1) at 560 nm (main).			
4	Chromogen	8	8	8
5	Mix and incubate for 5 minutes at room temp. Read the absorbance (OD2) at 560 nm (main).			

CALCULATION OF SAMPLE CONCENTRATION

$$\frac{(\text{OD2 sample} - \text{OD1 sample}) - (\text{OD2 blank} - \text{OD1 blank})}{(\text{OD2 calibrator} - \text{OD1 calibrator}) - (\text{OD2 blank} - \text{OD1 blank})} \times 200 = \text{Iron (µg/dL)}$$

Unit Conversion:

Iron (µg/dL) x 0.179 = Iron (µM)

Assay Example

	OD1 (560 nm)	OD2 (560 nm)	OD2-OD1	ΔOD	Iron (µg/dL)
Blank	0.031	0.033	0.002	-	-
Calibrator	0.028	0.139	0.111	0.109	-
Sample	0.042	0.101	0.059	0.057	104.6
Sample	104.6 µg/dL = 18.7 µM				

When assaying diluted samples, multiply the result by the dilution factor.

PERFORMANCE

Assay Range: 5 - 1,000 µg/dL

Precision: Precision was evaluated using commercially available quality control serum.

Within Run Precision	Mean (µg/dL)	S.D.	C.V.%
Level 1	111.18	1.05	0.9
Level 2	218.52	2.11	1.0

Interference:

Conjugated bilirubin	No interference up to at least 40 mg/dL
Unconjugated bilirubin	No interference up to at least 40 mg/dL
Hemoglobin	No interference up to at least 0.2 g/dL
Chyle	No interference up to at least 1,000 FTU

Shelf life: Until expiration date at 4℃. After opening any of the kit components, store at 4℃ and use within one month. **Do not freeze.**

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