Instruction manual

* FOR RESEARCH USE ONLY
* STORE AT 4℃ UPON ARRIVAL

Iron Assay kit LS

(Nitroso-PSAP Chromogenic method)

Description

This product is a direct colorimetric assay kit without deproteinization of the sample. Dissociated iron from the transferrin-iron complex by weakly acid buffer and reduced by means of reductant (:Ferric→Ferrous).Ferrous ions give a blue colored complex with Nitroso-PSAP (as chromogen). The intensity of the colored complex is proportional to the iron concentration in the sample. The color intensity is proportional to the amount of iron present in the sample.

Iron is an important element, which functions as an enzyme cofactor. All iron of in blood is bonded with transferrin, and they are transported to erythroblast or tissue for synthetic of globin protein which needs iron. Iron is an indispensable component to generate protein which transports oxygen. Its deficiency causes spanemia of hypoferrism, chronic hemorrhagic anemia and spanemia of infectivity. Increasing transferrin and high value of Iron can be observed in hepatitis or liver cirrhosis. Aplastic anemia and malignant anemia show increasing value of iron as well.

Kit contents

200 tests （Catalog # : FE02ME ）

R-A	Buffer 🔴	30 mL×1
R-B	Buffer 🔵	14 mL×1
R-R	Chelate color (Nitroso-PSAP) 🟡	1.5 mL×1
STD	Iron Standard 200 μg/dL 🟢	1.6 mL×1

Note

A) Unstableness of incubation temperature may result in unstable results.

B) Use disposable test tube and glassware washed with 1M HNO_3 or 1M HCl solution and distilled water.

C) Accuracy in pipetting volume for samples and reagents may affect the quality of assay. Please note that samples, standards and Working Reagent must be poured accurately μL level.

D) Temperature for chromogen reaction may affect optical density. Please try to extend or shorten chromogen reaction time depending on room temperature.

E) In the cell lysate or the tissue extract use as specimen, high concentration of proteins or lipid, may affect observed value. Please remove its by ultrafiltration or centrifugation.

F) Heme-containing iron species cannot be measured in this assay kit.

Operation

1. Sample preparation

◇Serum or Plasma

Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation. EDTA-plasma cannot be used.

◇Tissue extract, Lysate, Other samples.

Urine (24 hour pooled urine), or other biological fluid:

Add 6M HCl to the sample and adjust pH 2.0-3.0 (e.g. 5-10μL 6M HCl/ 1mL of lysate.). Centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use it for assay.

Tissue:

Add 5% TCA solution, vortex 1 min. and incubate at 4-8°C for 30 min. Centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use it for assay.

* Sample pH should be between pH2 to pH8.

2. Assay preparation

(1)Bring all reagents to room temperature before use.

(2)Prepare enough Working Reagent (WR).

		1 test	Example: 50 tests
R-B	Buffer 🔵	70 (μL)	3.5 (mL)
R-R	Chelate color 🟡	7 (μL)	350 (μL)

* WR is stored at 2-8°C and use within one month after prepared.

3. Assay procedure

(1 assay sample 250µL)

○Assay

(1) Add 15 µL of Distilled water (Blank) / STD (Standard)/ sample into each well.

(2) Add 160 µL of R-A Buffer to each well and incubate at room temperature for 10 min.

(3) Add 75 µL of Working Reagent (WR) to each well and incubate at room temperature for 5 min.

(4) Read the absorbance at 750 nm (main). --> OD

(Possible ranges of wavelength for selects the filter: 730-770 nm at 750nm)

		Assay Sample		
Add	(µL)	Blank OD_{Bl}	Standard OD_{Std}	Sample OD_S
1	Distilled water	15	-	-
	STD	-	15	-
	Assay sample	-	-	15
2	R-A	160	160	160
	↓ Mix and incubate for 10 minutes at room temperature			
3	WR	75	75	75
	↓ Mix and incubate for 5 minutes at room temperature Read the absorbance at 750 nm (main).			

○Calculations

$\Delta OD_{Std} = OD_{Std} - OD_{Bl}$, $\Delta OD_S = OD_S - OD_{Bl}$

Iron (µg/dL) = $\Delta OD_S/\Delta OD_{Std}$ X 200

Iron (µM) = $\Delta OD_S/\Delta OD_{Std}$ X 35.8

(Assay example)

	OD(750nm)	Δ OD	Iron (µg/dL)
Blank	0.055	-	-
Standard	0.118	0.063	-
Sample	0.089	0.034	107.9

Observed 750 nm only. [OD = OD(750nm)]

ΔOD_{Std} = 0.118 – 0.055 = 0.063

ΔOD_S = 0.089 - 0.055 = 0.034

$Iron_{Sample}$ (µg/dL) = $\Delta OD_S/\Delta OD_{Std}$ x 200 = 0.034/ 0.063 x 200
= 107.9 (µg/dL)

$Iron_{Sample}$ (µM) = $\Delta OD_S/\Delta OD_{Std}$ x 35.8 = 0.034/ 0.063 x 35.8
= 19.3 (µM)

*In diluted sample of seminal fluid, multiply the result by dilution-factor.

Performance

Measuring range 10 - 1000 µg/dL

Imprecision Imprecision was evaluated using commercially available quality control serum.

Within run

	Mean µg/dL	S.D	C.V %
Level 1	118.09	1.09	0.9
Level 2	244.73	8.89	3.6

Interferences No interference by the note of substances were observed.
Conjugated bilirubin and unconjugated bilirubin 40 mg/dL
Hemoglobin 0.1 g/dL Chyle 500 FTU

Expiration date and preservation conditions

Storage conditions: Store at 2-8°C. Don't freeze.

Expiration: 1 year from the date of manufacture. After the bottles are opened, the kit should be used in 1 month.

Reference

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Manufacturing-and-selling contractor

Metallogenics Co.,Ltd. Sales Dept.
1-8-15, Inohana, Chuo-ku Chiba-shi, Chiba, 260-0856, Japan
TEL : +81-43-227-6767
FAX : +81-43-227-6768
e-mail : sales@ak-j.com
URL: http://metallogenics.co.jp/