# Instruction manual

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

## Iron Assay kit LS

## ( Ferrozine 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 complex with Ferrozine (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 # : FE31ME ）

| | |
|---|---|
| R-A Buffer ● | 40 mL×1 |
| R-R Chelate color (Ferrozine) ● | 1.6 mL×1 |
| STD Iron Standard 200 µg/dL ● | 8.0 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 3% 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**

Bring all reagents to room temperature before use.

**3. Assay procedure**

**Procedure using microplate reader.**

**(1 assay sample 248µL)**

**○Assay**

(1) Add 200 µL of R-A to each well.

(2) Add 40 µL of Distilled water (Blank) / STD (Standard)/ sample into each well and incubate at room temperature for 5 min.

(3) Read the absorbance at 560 nm (main) --> OD1

(4) Add 8 µL of R-R to each well.

(5) Read the absorbance at 560 nm (main) --> OD2

| | | Assay Sample | | |
|---|---|---|---|---|
| Add | (µL) | Blank $OD_{Bl}$ | Standard $OD_{Std}$ | Sample $OD_S$ |
| 1 | R-A | 200 | 200 | 200 |
| 2 | Distilled water | 40 | | - |
| | STD | - | 40 | - |
| | Assay sample | - | - | 40 |
| | ↓ Mix and incubate for 5 minutes at room temperature. Read the absorbance at 560 nm. | | | |
| 3 | R-R | 8 | 8 | 8 |
| | ↓ Mix and incubate for 5 minutes at room temperature. Read the absorbance at 560 nm. | | | |

**○Calculations**

**$\Delta OD_{Std} = (OD2_{Std} – OD1_{Std}) – (OD2_{Bl} – OD1_{Bl})$**
**$\Delta OD_S = (OD2_S – OD1_S) – (OD2_{Bl} – OD1_{Bl})$**
**Iron (µg/dL) = $\Delta OD_S/\Delta OD_{Std}$ X 200**
**Iron (µM) = $\Delta OD_S/\Delta OD_{Std}$ X 35.8**

**(Assay example)**

| | OD1 (560nm) | OD2 (560nm) | OD | ΔOD | Iron (µg/dL) |
|---|---|---|---|---|---|
| Blank | 0.031 | 0.033 | 0.002 | - | - |
| Standard | 0.028 | 0.139 | 0.111 | 0.109 | - |
| Sample | 0.042 | 0.101 | 0.059 | 0.057 | 105 |

***Observed 560 nm**

**[OD = OD2(560nm) – OD1(560nm) ]**

$\Delta OD_{Std}$ = ( 0.139 - 0.028 ) – ( 0.033 - 0.031 ) = 0.109

$\Delta OD_S$ = ( 0.101- 0.042 ) – ( 0.033 - 0.031 ) = 0.057

Iron $_{Sample}$ (µg/dL) = $\Delta OD_S/\Delta OD_{Std}$ x 200
= 0.057/ 0.109 x 200 = 105 (µg/dL)

Iron $_{Sample}$ (µM) = $\Delta OD_S/\Delta OD_{Std}$ x 35.8
= 0.057/ 0.109 x 35.8 = 18.7 (µM)

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

**Performance**

Measuring range 5.0 - 1,000 µg/dL

Imprecision Imprecision was evaluated using commercially available quality control serum.

Within run

| | Mean µg/dL | S.D | C.V % |
|---|---|---|---|
| Level 1 | 111.18 | 1.05 | 0.9 |
| Level 2 | 218.52 | 2.11 | 1.0 |

Interferences No interference by the note of substances were observed.
Conjugated bilirubin and unconjugated bilirubin 40 mg/dL
Hemoglobin 0.2 g/dL Chyle 1,000 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**

1.) Lawrence L. Stookey , Analytical chemistry, VOL. 42, NO. 7,(1970)

2.) Nakaya. M, Tajima. M, Kosako. H, Nakaya. T, Hashimoto. A, Watari. K, Nishihara. H, Ohba. M, Komiya. S, Tani. N, Nishida. M, Taniguchi. H, Sato. Y, Matsumoto. M, Tsuda. M, Kuroda. M, Inoue. K, Kurose. H: GRK6 deficiency in mice causes autoimmune disease due to impaired apoptotic cell clearance, *Nature Commun*, 4, p1532 (2013).

3.) Ikeda. Y, Tajima. S, Izawa-Ishizawa. Y, Kihira. Y, Ishizawa. K, Tomita. S, Tsuchiya. K, Tamaki. T: Estrogen regulates hepcidin expression via GPR30-BMP6-dependent signaling in hepatocytes, *PLoS One*, 7(7) (2012).

4.) Tsugawa. H, Suzuki. H, Matsuzaki. J, Hirata. K, Hibi. T: FecA1, a bacterial iron transporter, determines the survival of Helicobacter pylori in the stomach, *Free Radic Biol Med*. 15, 52(6), p1003-10 (2012).

5.) Yasumasa. I, Hideaki. E, Soichiro. T, Yuki. I, Yoshitaka. K, Keisuke. I, Shuhei. T, Koichiro. T, Toshiaki. T: Dietary iron restriction inhibits progression of diabetic nephropathy in *db/db* mice, *American Journal of Physiology,* 304 p1028-1036 (2013)

6.) Yasumasa. I, Iori. O, Soichiro. T, Mizuki. I, Yuya. H, Yuki. I, Yoshitaka. K, Licht. M, Keisuke. I, Koichiro. T, Toshiaki. T: Iron Chelation by Deferoxamine Prevents Renal Interstitial Fibrosis in Mice with Unilateral Ureteral Obstruction, *PLoS One*, 9(2) (2014)

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