PRODUCT INFORMATION

Iron Assay (Nitroso-PSAP Chromogenic Method)

Cat. No. KT-756

INTENDED USE

The Iron Assay (Nitroso-PSAP 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 bound 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 blue chelate with the nitroso-PSAP chromogen. The intensity of this colored complex is proportional to the iron concentration in the sample and is measured at 750 nm.

COMPONENTS (~200 TESTS)

1. Buffer A, 30 mL x 1
2. Buffer B, 14 mL x 1
3. Chromogen (Nitroso-PSAP), 1.5 mL x 1
4. Iron Calibrator (200 µg/dL), 1.6 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 5% 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

1. Prepare enough Color Developer Solution for your experiment:

Color Developer Solution			
	1 test	50 tests	100 tests
Buffer B	70 µL	3.5 mL	7.0 mL
Chromogen	7 µL	350 µL	700 µL

   Color Developer Solution should be stored at 4℃ and used within one month after preparation.

2. Buffer A and the Iron Calibrator are ready to use.

3. Bring all reagents to room temperature before use.

ASSAY PROTOCOL (Microplate and Microplate Reader)

(Total reaction volume = 250 µL)

1. Add 15 µL of Blank (purified water), Iron Calibrator, or Sample to each well.

2. Add 160 µL of Buffer A to each well, mix, and incubate at room temperature for 10 minutes. Mix carefully using a pipette to avoid foaming. If a plate mixer is used for mixing, there is a risk of obtaining poor reproducibility.

3. Add 75 µL of prepared Color Developer Solution to each well, mix, and incubate at room temperature for 5 minutes. Mix carefully using a pipette to avoid foaming. If a plate mixer is used for mixing, there is a risk of obtaining poor reproducibility

4. Read the OD absorbance at 750 nm (main) using a plate reader or spectrophotometer. Acceptable wavelength range: 730 - 770 nm.

Assay Protocol				
Step	(µL)	Blank	Calibrator	Sample
1	Purified water	15	-	-
	Iron Calibrator	-	15	-
	Sample	-	-	15
2	Buffer A	160	160	160
	Mix and incubate for 10 minutes at room temp.			
3	Color Developer Solution	75	75	75
	Mix and incubate for 5 minutes at room temp.			
4	Read the OD absorbance at 750 nm (main).			

CALCULATION OF SAMPLE CONCENTRATION

$$\frac{\text{OD sample - OD blank}}{\text{OD calibrator - OD blank}} \times 200\ \mu g/dL\ \text{(calibrator value)} = \text{Iron } (\mu g/dL)$$

Unit Conversion:
Iron (µg/dL) x 0.179 = Iron (µM)

Assay Example

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

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

PERFORMANCE

Assay Range: 10 - 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	118.09	1.09	0.9
Level 2	244.73	8.89	3.6

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 0.1 g/dL
Chyle	No interference up to at least 500 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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