BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 955 Main St., Suite 4102, Buffalo, NY 14203
Tel: (716) 4586903 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Iron Assay Kit (Cat #: A-127)

COMPONENTS: Iron Assay Solution: 20 ml, store at 4°C (~150 wells; **avoid light exposure**)
Iron Probe: 50 mg, store at room temperature (RT)
Ascorbic Acid: 200 mg, store at room temperature (RT)
1 mM $FeCl_3$: 1 ml, store at room temperature (RT)

PRODUCT DESCRIPTION: The Iron Assay Kit is based on a modified ferene probing method and is suitable for analysis of iron present in cells/tissues and biological fluids such as serum/plasma, saliva, urine, and culture medium. The ferene-iron complex exhibits an absorbance max at 593-595 nm. The assay kit has a detection limit of 1-10 μM iron. Kit components are stable for several years if stored and handled properly.

$FeCl_3$ Standard Set:

Dilute 1 mM $FeCl_3$ 10-fold to 0.1 mM (100 μM), e.g., 0.1 ml 1 mM $FeCl_3$ + 0.9 ml dH_2O. Perform 1:1 serial dilution with dH_2O to obtain 50 μM, 25 μM, and 12.5 μM $FeCl_3$.

Sample preparation:

Serum/plasma: Mix 0.2 ml serum/plasma and 0.8 ml chloroform and vigorously vortex solution to ensure thorough mixing. Clarify solution by centrifugation at ~13,000 rpm for 10 min. Collect supernatant (top layer) for assay.

Cells/tissues: Freeze-thawed cells/tissues are mechanically homogenized in ice-cold dH_2O followed by centrifugation at ~13,000 rpm for 5 min to remove insoluble materials. Collect supernatant for assay.

Preparation of fresh ascorbic acid solution:

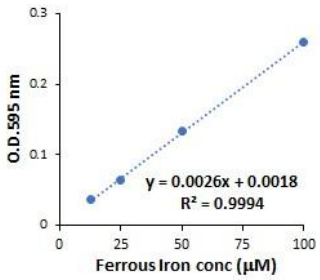
Prepare a fresh ascorbic acid solution by dissolving 20 mg Ascorbic Acid per ml of Iron Assay Solution. Each iron standard and sample require 20 μl and 40 μl ascorbic acid solution, respectively. Calculate the volume of ascorbic acid solution required for each assay. Use the solution immediately.

Preparation of fresh reaction solution:

Prepare a fresh reaction solution by dissolving 3 mg Iron Probe per ml of Iron Assay Solution. Each iron standard, each sample, and the H_2O well require 50 μl reaction solution. Calculate the volume of reaction solution required for assay. Use the solution immediately.

Iron assay:

1. Use a plain (uncoated) 96-well plate for the assay. Add 50 μl dH_2O to a well. Add 50 μl of each iron standard to a set of wells. Add 50 μl of each sample to duplicate wells serving as sample control and sample reaction wells.
2. Add 20 μl ascorbic acid solution to each well set up above. Agitate plate to mix contents briefly. Cover and incubate plate in a 37°C incubator for 30 min. *Do not use CO_2 incubator.*
3. Remove plate from incubator. Add 50 μl Iron Assay Solution to each sample control well. Add 50 μl reaction solution to the dH_2O well, each iron standard well, and each sample reaction well. Cover and incubate plate in a 37°C incubator for 10 min.
4. Read absorbance (O.D.) at 595 nm using a plate reader. Subtract dH_2O well reading from each iron standard well reading. Use the subtracted readings to generate an iron standard plot (see graph below; x= iron concentration in μM, y= $O.D._{595\ nm}$).
5. Subtract sample control well reading from sample reaction well reading for each sample. Use the subtracted readings for calculation of sample iron concentration as derived from the trend line equation of the standard plot.



NOTE:

- Samples should not contain the following chemicals: EDTA, sodium citrate, sodium fluoride, or oxalate.
- Please visit the product webpage or contact us for SDS information on acetic acid, NaOH, sodium acetate, ferene, and thiourea.