MA-0103

Iron Assay

(100 wells, Colorimetric, OD 593 nm, Store at 4°C)

Background Information:

Iron (MW 55.845 g/mol) is an essential element involved in a variety of metabolic processes in all known organisms. Most significant among these are electron transport, oxygen transport and DNA synthesis. The most common structures in which iron is found are e iron-sulfur centers and various hemes. Unlike most other ions whose levels are regulated by both absorption and excretion, iron levels in vivo are controlled solely by absorptive processes. AkrivisBio's Iron Assay is a simple direct method for determination of ferrous iron in samples. In the assay, iron compounds present in samples are optionally reduced to the ferrous form (Fe^{2+}), then reacted with Ferene S to produce a stable chromogen with λmax at 593 nm. Copper interference is prevented by the presence of an additive. The assay is useful in the range of 0 to 10 nanomoles iron in a variety of samples.

Assay Principle:

1 - Iron compounds are solubilized to make all Fe^{2+} and Fe^{3+} available. Cu^{2+} is chelated to remove interference.
2 - Reducing agent optionally added to convert Fe^{3+} to Fe^{2+} (total iron) or omitted (reduced iron, Fe^{2+} only).
3 - Ferene S is added to form the chromophore

Assay Components:

Assay buffer	25 ml	WM	MA-0103A
Iron Reducer	0.7 ml	Green	MA-0103B
Ferene S	12 ml	NM	MA-0103C
Fe^{3+} Standard, 100 mM	0.1 ml	Yellow	MA-0103D

Storage and Handling:

Store the kit at 4°C. Bring all components to room temperature before use. Mix the Iron Reducer to dissolve any precipitate that may have formed during freezing. Centrifuge all vials for a few seconds before opening.

Assay Protocol:

1. Iron Standard Curve: Dilute 10 µl of the 100 mM Iron Standard with 990 µl DI H_20 to generate a 1 mM Iron Standard. Add 0, 2, 4, 6, 8, and 10 µl of the diluted Iron Standard into a 96-well plate to generate 0, 2, 4, 6, 8, and 10 nmole/well Iron Standard. Add 5 µl Iron Reducer to each Standard well and bring the volume of all Standard wells to 100 µl with Iron Assay Buffer.

2. Sample Test:

a. Samples can be tested for ferrous (Fe^{2+}), or total (Fe^{2+} + Fe^{3+}) or ferric (Fe^{3+}) ion. Liquid samples such as serum can be tested directly (up to 50 µl of serum per well). Normal serum Iron levels are ~10-40 µM. Soft tissues or cells may be homogenized in 5-10 volumes of Iron Assay Buffer (*i.e.,* 500 µl Iron Assay Buffer for each 100 mg of wet tissue or ~5 x 10^6 cells). Thoroughly homogenize tissue samples with a probe sonicator or Dounce glass-bead homogenizer then centrifuge at 16,000 x *g* for 10 min and transfer the supernatant to a fresh microfuge tube. All final readings should be within the range of the Standard Curve. If any samples exceed that, it should be diluted appropriately and rerun.

b. For ferrous Iron (II) only assay: Add 1-50 µl sample to sample wells in a 96-well plate and bring the volume to 100 µl/well with Iron Assay Buffer.

For total Iron (II+III) assay: Add 1-50 µl sample to sample wells in a 96-well plate. Add 5 µl Iron Reducer to each sample to reduce Fe^{3+} to Fe^{2+}. Bring the volume to 100 µl/well with Iron Assay Buffer.

c. Allow for complete reduction to occur for 30 min at 37 °C of the Fe^{3+} to Fe^{2+}.

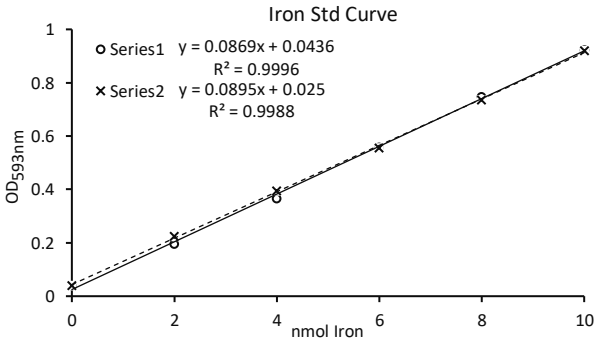
d. Add 100 µl Iron Probe to each well containing the Iron Standards and test samples. Mix well. Allow for full color development to occur for 60 min at 37°C, protected from light.

3. Measurement:

Monitor the absorbance at 593 nm in a microplate reader.

4. Typical Result:

nmole Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0412	0.0407	0	0
2	0.1958	0.2251	0.1546	0.1844
4	0.3670	0.3941	0.3258	0.3534
6	0.5573	0.5553	0.5161	0.5146
8	0.7482	0.7349	0.7070	0.6942
10	0.9240	0.9197	0.8828	0.8790



5. Calculation:

Subtract the zero Standard reading from all other standard and sample readings. Plot the Standard Curve. Determine the slope of the Standard Curve. Divide the background corrected sample readings by the slope of the Standard Curve to get nmoles of Iron in the well. Fe^{2+} and total iron (Fe^{2+} + Fe^{3+}) in test samples is determined directly from the Standard Curve. Fe^{3+} content of the test samples is calculated by subtracting Fe^{2+} from total iron (Fe^{2+} + Fe^{3+}). Determine the Fe^{2+}, Fe^{3+}, or total (Fe^{2+} + Fe^{3+}) concentration in the original samples by:

A. Divide the nmoles per well by the volume of sample added to the well in µl = nmoles Iron per µl sample
B. Multiply nmoles Iron per µl sample X the total volume of supernatant in 2a above = total nmoles Iron per sample
C. Divide total nmoles Iron per sample by the mg tissue or # of cells used to prepare the sample = nmoles Iron per mg tissue (or per # of cells, etc.)

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48511 Warm Springs Blvd. # 213, Fremont, CA 94539 +1(408)739-9315 contact@akrivisbio.com