Iron Assay Kit

Catalog Number KA0814

100 assays

Version: 04

Intended for research use only

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Table of Contents

Introduction

Background

Iron is essential to nearly all known organisms. It is generally stored in the centre of metalloproteins, in the heme complex, and in oxygen carrier proteins. Inorganic iron also contributes to redox reactions in the iron-sulfur clusters of many enzymes, such as nitrogenase and hydrogenase. The Iron Assay Kit provides a simple convenient means of measuring Ferrous and/or Ferric ion in sample. In the assay, ferric carrier protein will dissociate ferric into solution in the presence of acid buffer. After reduction to the ferrous form (Fe^{2+}), iron reacts with Ferene S to produce a stable colored complex and give absorbance at 593 nm. A specific chelate chemical is included in the buffer to block copper ion (Cu^{2+}) interference. The kit measures iron in the linear range of 0.4 to 20 nmol in 50 µL sample, or 8 µM to 400 µM iron concentration in various samples.

General Information

Materials Supplied

List of component

Component	Amount
Iron Assay Buffer	25 mL
Iron Probe	12 mL
Iron Reducer	0.7 mL
Iron Standard (100 mM)	0.1 mL

Storage Instruction

Store the kit at -20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge vials prior to opening. Read the entire protocol before performing the assay.

Assay Protocol

Assay Procedure

1. Standard curve:

   Dilute 10 µL of the 100 mM Iron Standard with 990 µL dH_2O to generate 1 mM standard Iron. 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 nmol/well standard. Bring the volume to 100 µL with Assay Buffer. Add 5 µL iron reducer to each standard well.

2. Sample test:

   Samples can be tested for ferrous (Fe^{2+}), or total Fe (II+III) or ferric (Fe^{3+}) ion. Liquid sample can be tested directly. Normal serum Iron ~10-40 µM. Tissue or cells can be lysed in 4-10 volume of Iron Assay Buffer, centrifuge 16000g for 10 min to remove insoluble materials. We suggest testing several doses of your samples to make sure the readings are within the standard curve range.

   - ✓ For the Iron (II) assay: Add 1-50 µL samples to sample wells in a 96-well plate and bring the volume to 100 µL/well with Assay Buffer. Add 5 µL Assay Buffer to each sample without Iron inducer.
   - ✓ For total Iron (II+III) assay: Add 1-50 µL samples to sample wells in a 96-well plate and bring the volume to 100 µL/well with Assay Buffer. Add 5 µL iron reducer to each sample to reduce Iron (III) to Iron (II).

3. Incubate iron standards and samples for 30 min at 25°C.
4. Add 100 µL Iron Probe to each well containing the iron standard and test samples. Mix well. Incubate the reaction for 60 min at 25°C, protect from light.
5. Measure the O.D. at 593 nm in a microplate reader.

Data Analysis

Calculation of Results

Subtract 0 standard reading from all standard and sample readings. Plot iron standard curve. Apply sample readings to the standard curve. Iron (II) and total iron (II+III) contents of the test samples can then be acquired directly from the standard curve. Iron (III) content of the test sample can be calculated by total iron (II+III) subtract iron (II). The iron (II), iron (III), and total iron (II+III) concentration in the samples can be calculated:

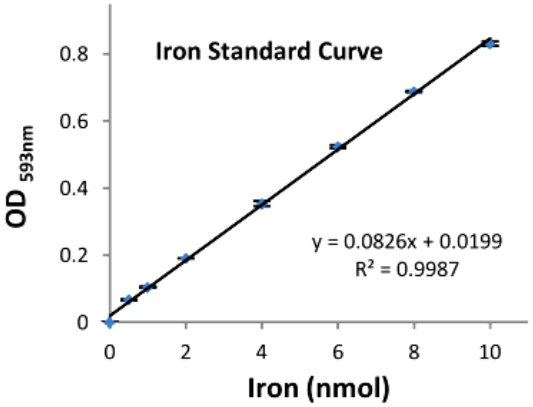
$$C = S_a / S_v \ (\text{nmol}/\mu\text{L}, \text{or mM})$$

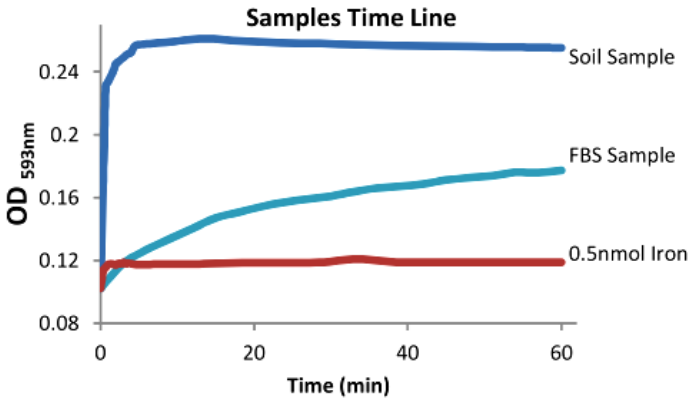
Where:

S_a is the iron (II), iron (III), or total iron (II+III) content of unknown samples (in nmol) from standard curve.

S_v is sample volume (µL) added into the assay wells.

Iron ion molecular weight is 55.845 g/mol.





Resources

Troubleshooting

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple freeze-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples, if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures until use
Lower/Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly

Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		