Product Information

Iron Assay Kit

Catalog Number MAK025
Storage Temperature –20 ºC

TECHNICAL BULLETIN

Product Description
Iron is a mineral that plays an essential role in many biological processes, including iron transport and redox reactions. Iron is a transition element that can form a range of oxidation states, the most common being iron II (Fe²⁺ or ferrous iron) and iron III (Fe³⁺ or ferric iron). Iron-containing proteins participate in many reactions, often utilizing transitory changes in the oxidation state of iron to carry out chemical reactions.

In this assay, iron is released by the addition of an acidic buffer. Samples may be tested directly to measure Fe²⁺ or reduced to measure total iron (Fe²⁺ and Fe³⁺). Released iron is reacted with a chromagen resulting in a colorimetric (593 nm) product, proportional to the iron present. The Iron Assay Kit provides a simple convenient means of measuring iron in a variety of biological samples.

Components
The kit is sufficient for 100 assays in 96 well plates.

Iron Assay Buffer 25 mL
Catalog Number MAK025A

Iron Probe 12 mL
Catalog Number MAK025B

Iron Reducer 0.7 mL
Catalog Number MAK025C

Iron Standard, 100 mM 0.1 mL
Catalog Number MAK025D

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate. It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Iron Assay Buffer- Allow buffer to come to room temperature before use.

Storage/Stability
The kit is shipped on wet ice and storage at –20 ºC, protected from light, is recommended.

Procedure
Iron Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM Iron Standard with 990 µL of water to generate a 1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM standard solution into a 96 well plate to generate 0, 2, 4, 6, 8, and 10 nmole/well standards. Add Iron Assay Buffer to each well to bring the volume to 100 µL. Add 5 µL of the Iron Reducer to each standard well.

Sample Preparation
Tissue (10 mg) or cells (2 × 10⁶) should be rapidly homogenized in 4–10 volumes of Iron Assay buffer. Centrifuge at 13,000 × g for 10 minutes at 4 ºC to remove insoluble material.

Serum and other liquid samples can be directly added to the wells. This kit is not suitable for use with plasma samples.

Bring samples to a final volume of 100 µL with Iron Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.
Assay Reaction

1. This assay can be used to measure either ferrous (Fe$^{2+}$) iron, total iron, or ferric (Fe$^{3+}$) iron (total iron – ferrous iron).
   - To measure ferrous iron, add 5 μL of iron assay buffer to each well.
   - To measure ferric iron, set up two sets of wells. Add 5 μL of assay buffer to the samples in one set of wells and 5 μL of Iron Reducer to the other set of wells.
   - To measure total iron, add 5 μL of Iron Reducer to each of the sample wells to reduce Fe$^{3+}$ to Fe$^{2+}$.

2. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at room temperature. Protect the plate from light during the incubation.

3. Add 100 μL of Iron Probe to each well containing standard or test samples. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 60 minutes at room temperature. Protect the plate from light during the incubation.

4. Measure the absorbance at 593 nm ($A_{593}$).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) iron Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate iron standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Concentration of Iron

$\frac{S_a}{S_v} = C$

$S_a$ = Amount of iron in unknown sample (nmole) from standard curve
$S_v$ = Sample volume (μL) added into the wells
$C$ = Concentration of iron in sample

Fe$^{2+}$ and total iron (Fe$^{2+}$ + Fe$^{3+}$) concentrations can be determined from the standard curve. Fe$^{3+}$ is equal to total iron (sample plus iron reducer) – Fe$^{2+}$ (sample plus assay buffer).

Iron atomic mass is 55.85 g/mole

Sample Calculation

Amount of iron ($S_a$) = 5.84 nmole
   (from standard curve)
Sample volume ($S_v$) = 100 μL

Concentration of iron in sample

$\frac{5.84 \text{ nmole}}{100 \mu L} = 0.0584 \text{ nmole/μL}$

$0.0584 \text{ nmole/μL} \times 55.85 \text{ ng/nmole} = 3.26 \text{ ng/μL}$
## Troubleshooting Guide

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<td>Assay Buffer Ice Cold</td>
<td>Assay Buffer must be at room temperature</td>
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<td>Check filter settings of instrument</td>
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<td>Samples prepared in different buffer</td>
<td>Use the Iron Assay Buffer</td>
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<td>Repeat the sample homogenization, increasing the length and extent of homogenization step.</td>
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<td>Samples used after multiple freeze-thaw cycles</td>
<td>Aliquot and freeze samples if needed to use multiple times</td>
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<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples and store correctly until use</td>
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<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
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<td>Use of expired kit or improperly stored reagents</td>
<td>Always check the expiration date and store the components appropriately</td>
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<td>Allowing the reagents to sit for extended times on ice</td>
<td>Always prepare fresh reaction mix before use</td>
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<td>Refer to Technical Bulletin and verify correct incubation times and temperatures</td>
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<td>Incorrect volumes used</td>
<td>Use calibrated pipettes and aliquot correctly</td>
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<td>Non-linear Standard Curve</td>
<td>Use of partially thawed components</td>
<td>Thaw and resuspend all components before preparing the reaction mix</td>
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<td>Prepare a master reaction mix whenever possible</td>
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<td>Pipette gently against the wall of the tubes</td>
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<td>Standard stock is at incorrect concentration</td>
<td>Always refer to the dilutions in the Technical Bulletin</td>
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<td>Calculation errors</td>
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<td>Substituting reagents from older kits/ lots</td>
<td>Use fresh components from the same kit</td>
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<td>Unanticipated results</td>
<td>Samples measured at incorrect wavelength</td>
<td>Check the equipment and filter settings</td>
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<td>Samples contain interfering substances</td>
<td>If possible, dilute sample further</td>
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<td>Sample readings above/below the linear range</td>
<td>Concentrate or dilute samples so that it is in the correct linear range</td>
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