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Product Information

Iron Assay Kit

Catalog Number **MAK025**
Storage Temperature –20 °C

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 (Fe^{2+} or ferrous iron) and iron III (Fe^{3+} 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 Fe^{2+} or reduced to measure total iron (Fe^{2+} and Fe^{3+}). 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 Catalog Number MAK025A	25 mL
Iron Probe Catalog Number MAK025B	12 mL
Iron Reducer Catalog Number MAK025C	0.7 mL
Iron Standard, 100 mM Catalog Number MAK025D	0.1 mL

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 °C, 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 (2×10^6) should be rapidly homogenized in 4–10 volumes of Iron Assay buffer. Centrifuge at 16,000 × *g* for 10 minutes at 4 °C 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 1–50 μL samples to sample wells in a 96 well plate and bring the volume to 100 μL per well with Assay Buffer. Add 5 μL of iron assay buffer to each sample.
   - To measure total iron, add 1–50 μL samples to sample wells in a 96 well plate and bring the volume to 100 μL per well with Assay Buffer. 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 25 °C. Protect the plate from light during the incubation.
3. Add 100 μL of Iron Probe to each well containing standard and test samples. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 60 minutes at 25 °C. 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

$$S_a/S_v = C$$

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

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

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

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Assay Buffer Ice Cold	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Iron Assay Buffer
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/Higher Readings in Samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Always check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Always prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear Standard Curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the reaction mix	Prepare a master reaction mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to the dilutions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so that it is in the correct linear range

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