Technical Bulletin

Iron Assay Kit

Catalogue Number MAK472

Product Description

Iron level in blood is a reliable diagnostic indicator of various disease states. Increased levels of iron concentration in blood are associated with red blood cell destruction, decreased blood cell survival, acute hepatitis, certain sideroblastic anemias, ingestion of iron-rich diets, defects in iron storage (for example, pernicious anemia). Decreased levels of blood iron may result from insufficient iron ingestion from diets, chronic blood loss pathologies, or increased demand on iron storage as during normal pregnancy.

Simple, direct and automation-ready procedures for measuring iron concentrations have applications in research, drug discovery and environmental monitoring. The Iron Assay Kit is designed to measure total iron directly in serum without pretreatment. The improved method utilizes a chromogen that forms a blue colored complex specifically with Fe^{2+}. Fe^{3+} in the sample is reduced to Fe^{2+}, allowing the assay to determine total iron concentration. The intensity of the color, measured at 590 nm, is directly proportional to the iron concentration in the sample.

The linear detection range of the kit is 27 to 1,000 µg/dL (4.8 µM to 179 µM) iron in a 96-well plate assay. The kit is suitable for the detection of iron in biological samples such as serum and soil extracts, and for studying the effects of drugs on iron metabolism.

Components

The kit is sufficient for 250 colorimetric assays in 96-well plates.

- Reagent A — 50 mL
  Catalogue Number MAK472A
- Reagent B — 4 mL
  Catalogue Number MAK472B
- Reagent C — 4 mL
  Catalogue Number MAK472C
- Iron Standard (10 mg/dL Fe^{2+}) — 1 mL
  Catalogue Number MAK472D

Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at 2-8 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Procedure

All Samples and standards should be run in duplicate.

Iron chelators (such as EDTA) interfere with this assay and should be avoided in Sample preparation.

Sample Preparation

1. Serum or plasma Samples should be clear and free of precipitates or turbidity. If turbidity is present, centrifuge or filter to clarify samples prior to assay.
2. Transfer 50 µL of Sample into a clear flat bottom 96-well plate.
3. For serum and plasma Samples, transfer an additional 50 µL of Sample into a separate well for use as a Sample Blank.

Standard Curve Preparation

1. Prepare a 1000 µg/dL Iron Standard by mixing 40 µL of the 10 mg/dL Iron standard with 360 µL purified water.
2. Prepare standards in 1.5-mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Iron Standards

Well	1000 µg/dL Standard	Purified Water	Iron (µg/dL)
1	100 µL	-	1000
2	80 µL	20 µL	800
3	60 µL	40 µL	600
4	40 µL	60 µL	400
5	30 µL	70 µL	300
6	20 µL	80 µL	200
7	10 µL	90 µL	100
8	-	100 µL	0

3. Mix well and transfer 50 µL of each Standard into separate wells of the plate.

Working Reagent

1. Mix enough reagents for the number of assays to be performed. For each Sample and Standard well, prepare Working Reagent according to Table 2.
   Note: Fresh preparation of Working Reagent is recommended. Equilibrate to room temperature prior to assay.

Table 2.
Preparation of Working Reagent

Reagent	Volume
Reagent A	200 µL
Reagent B	10 µL
Reagent C	10 µL

2. Add 200 µL of Working Reagent to each Standard and Samples well.
3. For serum or plasma Samples which require a Sample Blank, add 200 µL of Reagent A to each Sample Blank well.
4. Tap plate to mix.

Measurement

1. Incubate plate for 40 minutes at room temperature.
2. Read optical density (OD) at 590 nm.

Results

1. Calculate ΔOD by subtracting the blank OD reading of Standard #8 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD against standard concentrations and determine the slope of the standard curve using linear regression fitting.

3. Calculate the iron concentration of the sample:

Iron (µg/dL) =

$$\frac{OD_{Sample} - OD_{blank}}{Slope}$$

where:

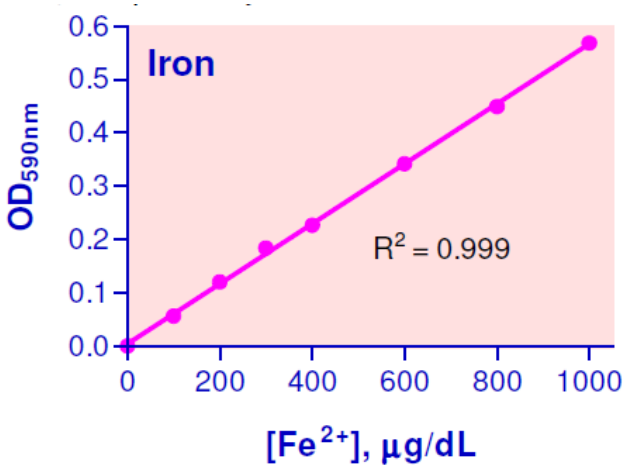
R_{Sample} = OD reading of Sample

R_{Blank} = OD reading of Blank (Standard #8), or Sample Blank, if applicable (if testing serum or plasma).

Typical serum iron values: 70-180 µg/dL.

Conversions: 1 mg/dL iron equals 179 µM, 0.001% or 10 ppm.

Typical Iron Standard Curve



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