

## 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  $\text{Fe}^{2+}$ .  $\text{Fe}^{3+}$  in the sample is reduced to  $\text{Fe}^{2+}$ , 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  $\mu\text{g/dL}$  (4.8  $\mu\text{M}$  to 179  $\mu\text{M}$ ) 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 $\text{Fe}^{2+}$ ) | 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  $\mu\text{L}$  of Sample into a clear flat bottom 96-well plate.
3. For serum and plasma Samples, transfer an additional 50  $\mu\text{L}$  of Sample into a separate well for use as a Sample Blank.

### Standard Curve Preparation

1. Prepare a 1000  $\mu\text{g}/\text{dL}$  Iron Standard by mixing 40  $\mu\text{L}$  of the 10  $\text{mg}/\text{dL}$  Iron standard with 360  $\mu\text{L}$  purified water.
2. Prepare standards in 1.5-mL microcentrifuge tubes according to Table 1.

**Table 1.**

Preparation of Iron Standards

Well	1000 $\mu\text{g}/\text{dL}$ Standard	Purified Water	Iron ( $\mu\text{g}/\text{dL}$ )
1	100 $\mu\text{L}$	-	1000
2	80 $\mu\text{L}$	20 $\mu\text{L}$	800
3	60 $\mu\text{L}$	40 $\mu\text{L}$	600
4	40 $\mu\text{L}$	60 $\mu\text{L}$	400
5	30 $\mu\text{L}$	70 $\mu\text{L}$	300
6	20 $\mu\text{L}$	80 $\mu\text{L}$	200
7	10 $\mu\text{L}$	90 $\mu\text{L}$	100
8	-	100 $\mu\text{L}$	0

3. Mix well and transfer 50  $\mu\text{L}$  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 $\mu\text{L}$
Reagent B	10 $\mu\text{L}$
Reagent C	10 $\mu\text{L}$

2. Add 200  $\mu\text{L}$  of Working Reagent to each Standard and Samples well.
3. For serum or plasma Samples which require a Sample Blank, add 200  $\mu\text{L}$  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  $\Delta\text{OD}$  by subtracting the blank OD reading of Standard #8 (Blank) from the remaining Standard reading values.
2. Plot the  $\Delta\text{OD}$  against standard concentrations and determine the slope of the standard curve using linear regression fitting.

3. Calculate the iron concentration of the sample:

Iron ( $\mu\text{g/dL}$ ) =

$$\frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{blank}}}{\text{Slope}}$$

where:

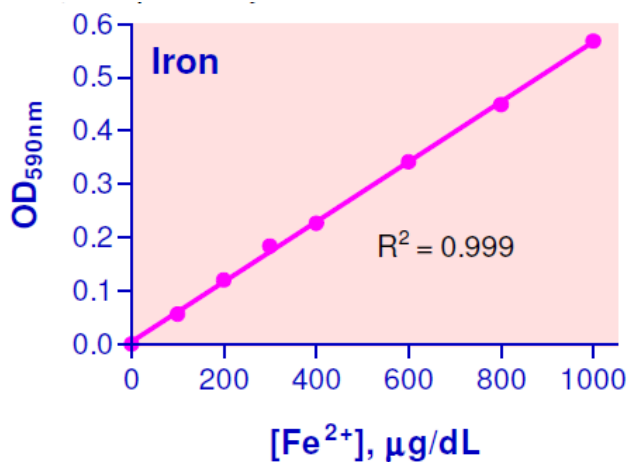
$R_{\text{Sample}}$  = OD reading of Sample

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

Typical serum iron values: 70-180  $\mu\text{g/dL}$ .

Conversions: 1 mg/dL iron equals 179  $\mu\text{M}$ , 0.001% or  
10 ppm.

Typical Iron Standard Curve



---

## Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

## Technical Assistance

Visit the tech service page at [SigmaAldrich.com/techservice](https://SigmaAldrich.com/techservice).

## Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at [SigmaAldrich.com/terms](https://SigmaAldrich.com/terms).

## Contact Information

For the location of the office nearest you, go to [SigmaAldrich.com/offices](https://SigmaAldrich.com/offices).

The life science business of Merck operates  
as MilliporeSigma in the U.S. and Canada.

Merck and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates.  
All other trademarks are the property of their respective owners. Detailed information on  
trademarks is available via publicly accessible resources.

© 2024 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

mak472pis Rev 04/24

