Technical Bulletin

Total Iron Assay Kit

Catalogue Number MAK606

Product Description

The Total Iron Assay Kit is suitable for quantitively measuring low concentrations of ferrous iron (50-1000 nanograms). The assay is based on measuring the absorbance of the iron probe (absorbing at 593 nm) that is formed in the reaction. The kit directly measures ferrous iron (Fe^{2+}) which binds to the probe to give a stable deep blue color. To measure ferric iron (Fe^{3+}), the ion needs to be reduced to Fe^{2+} with the supplied reducer, which can then be detected by the probe. Enabling the distinguishment between ferric and ferrous iron [1,2].

The assay is linear over the range of 50-1000 ng. In the standard solutions, the reaction is virtually instantaneous however, in biological samples the reduction of ferric iron may take a considerable time. The kit is suitable for the measurement of iron in various biological samples, including blood serum, tissues and cells. In serum, there may be contamination of the sample with copper ions which can also react with the reagent, therefore thiourea is added to the assay buffer[3].

References

1. Hennessy DJ et al, (1984) *Can. J. Chem.*, ***62***, 721-724
2. Smith GL et al, (2021) *J. Inorg. Biochem.*, ***220***, 111460
3. Artiss JD et al, (1981) *Clin. Biochem.*, ***14***, 311-315

Components

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

- Assay Buffer — 30 mL
  Catalogue number MAK606A
- Iron Probe — 1.5 mL
  Catalogue number MAK606B
- Iron Reducer — 0.78 g
  Catalogue number MAK606C
- Iron Standard Solution — 0.5 mL
  Catalogue number MAK606D

Reagents and Equipment Required but Not Provided

- 96-well flat-bottom plate.
  - Clear plates for colorimetric assays (Catalogue number M2936 or equivalent)
  - Cell culture or tissue culture treated plates are not recommended.
- Plate reader that is able to read absorbance at 593 nm.
- Pipettors and Pipettes
- Vortex Mixer
- Triton™ X-100, 10% solution (Catalogue number 93443 or equivalent).

Precautions and Disclaimer

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.

Storage/Stability

The product is shipped at ambient temperature. Store at Store at 2-8°C upon receipt.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Assay Buffer (MAK606A)

Allow buffer to come to room temperature before use.

Iron Reducer (MAK606B)

Reconstitute the Iron Reducer by adding 7.8 mL of water to the bottle.

Preparation of Reaction Mixture

To each 1 mL of Assay Buffer MAK606A add 50 µl Iron Probe MAK606B and 150 µl Iron Reducer MAK606C. Mix by inversion.

Procedure

All samples and standards should be run in at least duplicates. A convenient customer calculator can be found on the product webpage.

Preparation of Iron Standards

Dilute 10 µL of the 1 mg/mL standard in 990 µl water to create a 10 µg/mL solution. Mix well be pipetting up and down.

Prepare 6 standards by mixing the diluted standard with the assay buffer in the wells of a 96-well plate, according to Table 1.

Std #	10 µg/ml standard (µL)	Assay Buffer (µL)	Concentration (ng/well)
1	0	50	0
2	10	40	100
3	20	30	200
4	30	20	300
5	40	10	400
6	50	0	500

Sample Preparation

Serum or Plasma Samples

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. For total iron determination: Preincubate samples with the iron reducer (30 µl reducer per 250 µl sample) for 30 minutes at room temperature. The iron released from transferrin in serum is ferric iron, and this needs to be reduced to ferrous iron for the assay.

   Note: If the iron reducer is added after the iron probe, the color appearance will be very slow (several hours).
3. If the ratio of ferric iron to ferrous iron is desired, the assay should be performed in two parallel steps. A) for ferrous iron the preincubation step should be eliminated B) for total iron the preincubation step should be performed.
4. Transfer 25 and 50 µL of Sample into a clear flat bottom 96-well plate and then proceed to the reaction. **It is always recommended to perform the test on two volumes of samples (5-50 µL).**
5. Read at 593 nm.

Cell Cultures

1. Grow a culture on a 75 cm^2 dish until at least 80% confluence. Remove the cells with Trypsin-EDTA solution from the plate and suspend in PBS. Centrifuge the samples at 10,000 × g for 10 minutes to remove insoluble material and resuspend the cells in PBS.
2. Take 1000 µl of cell suspension ($8x10^6$ cells) and add 115 µl of 10% Triton X-100. Incubate at 37°C for at least 30

minutes for dissolution of the cell membrane.

3. Take aliquots of 250 µl and add 38 µl of the reducer solution. (There are approximately $2x10^6$ cells per 250 µl sample). Incubate at room temperature for 45 minutes and then proceed to the reaction.
4. Read at 593 nm.

Assay Reaction

1. Add the standard or sample to each well in duplicate. For samples, complete the volume to 50 µl with DDW.
2. Add 200 µl of Reaction mixture to each well with a multipipette and mix by pipetting.
3. Read the results in the plate reader at 593 nm.

Results

Calculations

The background for the assays is the value obtained for the zero ferrous iron standard. Correct for the background by subtracting the blank standard value from all readings. Input the corrected values of the ferrous iron standards into the **Customer Calculator** Excel sheet or any other spreadsheet in order to plot a standard curve and extract the linear equation.

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

Subtract the blank sample value from the sample reading in order to obtain the corrected absorbance measurement. Calculate the amount of ferrous iron in the sample according to the standard curve's line equation.

The final concentration of ferrous iron can be calculated according to the following equation:

$$\text{Iron in sample (ng/ml)} = \frac{\mathbf{C \times DF}}{\mathbf{V}}$$

where:
C = amount of iron as determined by the linear equation of the calibration curve (ng)
V = Sample volume (mL) added into the wells.
DF = dilution factor

To convert the result from ng/mL to µg/dL for serum samples:

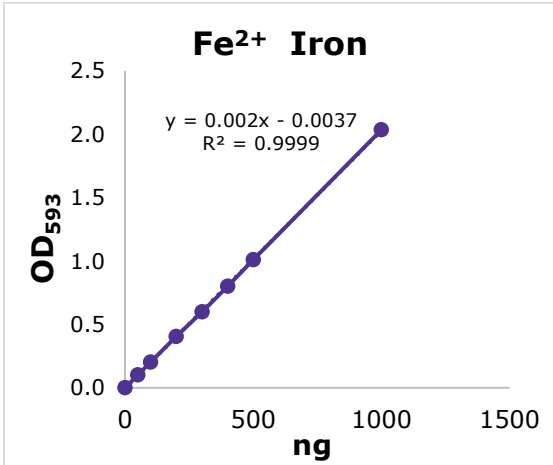
$$\mu g/dL = \frac{(ng/ml)x100}{1000}$$

Where 100 = 100 ml = 1 deciliter, and 1000 = 1000 ng per microgram.

Typical serum iron values: 60-180 µg/dL.

Figure 1.

Exemplary Standard Curve



Note: the normal standard curve is measured from 100-500 ng, however the assay can be measured over a larger range of 50-1000 ng.

References

1. Hennessy DJ et al, (1984) *Can. J. Chem*., ***62***, 721-724
2. Smith GL et al, (2021) *J. Inorg. Biochem*., ***220***, 111460
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