

# Iron Assay Kit

(Catalog #K390-100; 100 Reactions; Store kit at -20°C)

## I. Introduction:

Iron is essential to nearly all known organisms. It is generally stored in the centre of metalloproteins, in the heme complex, and in oxygen carrier proteins. Inorganic iron also contributes to redox reactions in the iron-sulfur clusters of many enzymes, such as nitrogenase and hydrogenase. BioVision's Iron Assay Kit provides a simple convenient means of measuring Ferrous and/or Ferric ion in sample. In the assay, ferric carrier protein will dissociate ferric into solution in the presence of acid buffer. After reduction to the ferrous form (Fe<sup>2+</sup>), iron reacts with Ferene S to produce a stable colored complex and give absorbance at 593 nm. A specific chelate chemical is included in the buffer to block copper ion (Cu<sup>2+</sup>) interference. The kit measures iron in the linear range of 0.4 to 20 nmol in 50 µl sample, or 8 µM to 400 µM iron concentration in various samples.

## II. Kit Contents:

Components	K390-100	Cap Code	Part No.
Iron Assay Buffer	15 ml	WM	K390-100-1
Iron Probe	12 ml	NM	K390-100-2
Iron Reducer	0.7 ml	Green	K390-100-3
Iron Standard (100 mM)	0.1 ml	Yellow	K390-100-4

## III. Storage and Handling:

Store the kit at -20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge vials prior to opening. Read the entire protocol before performing the assay.

## IV. Iron Assay Protocol:

### 1. Standard curve:

Dilute 10 µl of the 100 mM Iron Standard with 990 µl dH2O to generate 1 mM standard Iron. Add 0, 2, 4, 6, 8, and 10 µl of the diluted Iron standard into a 96-well plate to generate 0, 2, 4, 6, 8, and 10 nmol/well standard. Bring the volume to 100 µl with Assay Buffer. Add 5 µl iron reducer to each standard well.

### 2. Sample test:

Samples can be tested for ferrous (Fe<sup>2+</sup>), or total Fe(II+III) or ferric (Fe<sup>3+</sup>) ion. Liquid sample can be tested directly. Normal serum Iron ~10-40 µM. Tissue or cells can be lysed in 4-10 volume of Iron Assay Buffer, centrifuge 16000g for 10 min to remove insoluble materials. We suggest testing several doses of your samples to make sure the readings are within the standard curve range.

**For the Iron (II) assay:** Add 1-50 µl samples to sample wells in a 96-well plate and bring the volume to 100 µl/well with Assay Buffer. Add 5 µl Assay Buffer to each sample without Iron inducer.

**For total Iron (II+III) assay:** Add 1-50 µl samples to sample wells in a 96-well plate and bring the volume to 100 µl/well with Assay Buffer. Add 5 µl iron reducer to each sample to reduce Iron (III) to Iron (II).

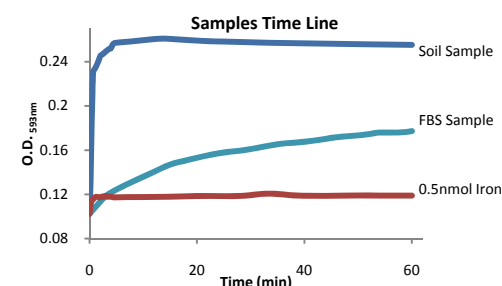
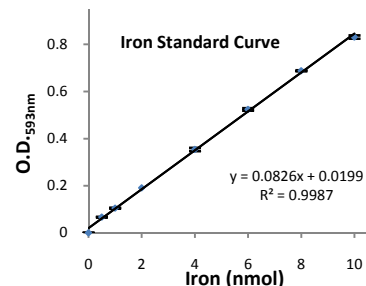
- Incubate iron standards and samples for 30 min at 25°C.
- Add 100 µl Iron Probe to each well containing the iron standard and test samples. Mix well. Incubate the reaction for 60 min at 25°C, protect from light.
- Measure the O.D. at 593 nm in a microplate reader.
- Calculation:** Subtract 0 standard reading from all standard and sample readings. Plot iron standard curve. Apply sample readings to the standard curve. Iron (II) and total iron (II+III) contents of the test samples can then be acquired directly from the standard curve. Iron (III) content of the test sample can be calculated by total iron (II+III) subtract iron (II). The iron(II), iron(III), and total iron(II+III) concentration in the samples can be calculated:

$$C = S_a/S_v \text{ (nmol/}\mu\text{l, or mM)}$$

where **S<sub>a</sub>** is the iron (II), iron (III), or total iron (II+III) content of unknown samples (in nmol) from standard curve.

**S<sub>v</sub>** is sample volume (µl) added into the assay wells.

Iron ion molecular weight is 55.845 g/mol.



## V. Related Products:

- NAD(P)/NAD(P)H Quantification Kit
- FAD Assay Kit
- Ascorbic Acid Quantification Kit
- Total Antioxidant Capacity (TAC) Assay Kit
- Ethanol Assay Kit
- Pyruvate Assay Kit
- Creatinine Assay Kit
- Ammonia Assay Kit
- Triglyceride Assay Kit
- Choline/Acetylcholine Quantification Kit
- Sarcosine Assay Kit
- Glycogen Assay Kit
- Creatinine Assay Kit
- Creatine Assay Kit
- Urea Assay Kit
- Phosphatase Assay Kit

- ADP/ATP Ratio Assay Kit
- CoA Assay Kit
- Glutathione Detection Kit
- Fatty Acid Assay Kit
- Uric Acid Assay Kit
- Lactate Assay Kit I & II
- Nitric Oxide Assay Kit
- Free Glycerol Assay Kit
- Hemin Assay Kit
- Glucose Assay Kit
- L-Amino Acid Assay Kit
- Cholesterol Assay Kit
- HDL & LDL Assay Kit
- Fatty Acid Assay Kit
- Ammonia Assay Kit
- Phosphate Assay Kit