

## Iron Colorimetric Assay Kit

(Catalog #K390-100; 100 Reactions; Store kit at  $-20^{\circ}\text{C}$ )

### I. Introduction:

Iron is essential to nearly all known organisms. It is generally stored in the center 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 samples. In the assay, ferric carrier protein will dissociate ferric ions into solution in the presence of acidic buffer. After reduction to the ferrous form ( $\text{Fe}^{2+}$ ), 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 ( $\text{Cu}^{2+}$ ) interference. The kit measures iron in the linear range of 0.4 to 20 nmol in 50  $\mu\text{l}$  sample, or 8  $\mu\text{M}$  to 400  $\mu\text{M}$  iron concentration in various samples.

### II. Kit Contents:

Components	K390-100	Cap Code	Part No.
Iron Assay Buffer	25 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  $-20^{\circ}\text{C}$ , protect from light. Warm Assay Buffer to room temperature before use. Mix the Iron Reducer to dissolve any precipitate that may have formed during freezing. Briefly centrifuge vials prior to opening. Read the entire protocol before performing the assay.

### IV. Iron Assay Protocol:

#### 1. Standard curve:

Dilute 10  $\mu\text{l}$  of the 100 mM Iron Standard with 990  $\mu\text{l}$  dH<sub>2</sub>O to generate 1 mM Iron Standard. Add 0, 2, 4, 6, 8, and 10  $\mu\text{l}$  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  $\mu\text{l}$  with Assay Buffer. Add 5  $\mu\text{l}$  Iron Reducer to each standard well.

#### 2. Sample test:

Samples can be tested for ferrous ( $\text{Fe}^{2+}$ ), or total  $\text{Fe(II+III)}$  or ferric ( $\text{Fe}^{3+}$ ) ion. Liquid samples can be tested directly. Normal serum Iron is  $\sim 10\text{-}40\ \mu\text{M}$ . Tissue or cells can be lysed in 4-10 volumes of Iron Assay Buffer and then centrifuged at 16000g for 10 min to remove insoluble material. 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  $\mu\text{l}$  samples to sample wells in a 96-well plate and bring the volume to 100  $\mu\text{l}$ /well with Iron Assay Buffer. Add 5  $\mu\text{l}$  Iron Assay Buffer to each sample without Iron Reducer.

**For total Iron (II+III) assay:** Add 1-50  $\mu\text{l}$  samples to sample wells in a 96-well plate and bring the volume to 100  $\mu\text{l}$ /well with Assay Buffer. Add 5  $\mu\text{l}$  Iron Reducer to each sample to reduce iron (III) to iron (II).

- Incubate iron standards and samples for 30 min at  $37^{\circ}\text{C}$ .
- Add 100  $\mu\text{l}$  Iron Probe to each well containing the iron standard and test samples. Mix well. Incubate the reaction for 60 min at  $37^{\circ}\text{C}$ , 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 the 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 determined directly from the standard curve. Iron (III) content of the test samples can be calculated by subtracting iron (II) from total iron (II+III). The iron(II), iron(III), and total iron(II+III) concentration in the samples can be calculated as follows:

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

where  $S_a$  is the iron (II), iron (III), or total iron (II+III) content of unknown samples (in nmol) from standard curve.

$S_v$  is the sample volume ( $\mu\text{l}$ ) added into the assay wells.

Iron ion molecular weight is 55.845 g/mol.

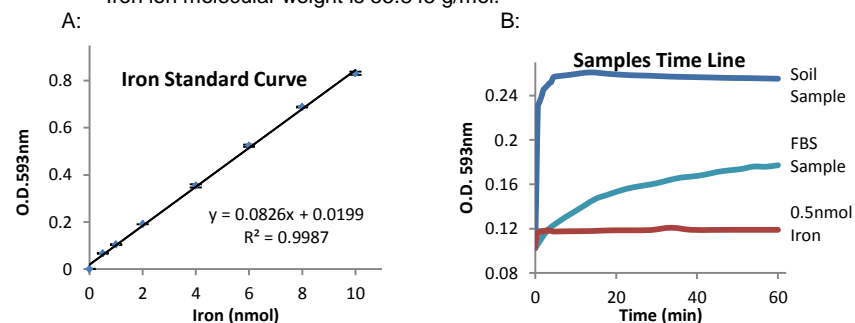


Figure: A: Iron Standard Curve. B: Assay of soluble free iron from a soil sample ( 5  $\mu\text{l}$  of 100  $\mu\text{l}$  buffer into which 100 mg of soil has been stirred), 5  $\mu\text{l}$  of FBS and 5  $\mu\text{l}$  of a 100  $\mu\text{M}$  sample of iron standard.

### RELATED PRODUCTS:

NAD(P)/NAD(P)H Quantification Kit  
 Ascorbic Acid Quantification Kit  
 Total Antioxidant Capacity (TAC) Assay Kit  
 Ethanol Assay Kit  
 Pyruvate 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

ADP/ATP Ratio Assay Kit  
 Glutathione Detection Kit  
 Fatty Acid Assay Kit  
 Uric Acid Assay Kit  
 Lactate Assay Kit I & II  
 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

**FOR RESEARCH USE ONLY! Not to be used on humans.**

## GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> <li>• Use of ice-cold assay buffer</li> <li>• Omission of a step in the protocol</li> <li>• Plate read at incorrect wavelength</li> <li>• Use of a different 96-well plate</li> </ul>	<ul style="list-style-type: none"> <li>• Assay buffer must be at room temperature</li> <li>• Refer and follow the data sheet precisely</li> <li>• Check the wavelength in the data sheet and the filter settings of the instrument</li> <li>• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates</li> </ul>
Samples with erratic readings	<ul style="list-style-type: none"> <li>• Use of an incompatible sample type</li> <li>• Samples prepared in a different buffer</li> <li>• Samples were not deproteinized (if indicated in datasheet)</li> <li>• Cell/ tissue samples were not completely homogenized</li> <li>• Samples used after multiple free-thaw cycles</li> <li>• Presence of interfering substance in the sample (e.g. metal ion chelators)</li> <li>• Use of old or inappropriately stored samples</li> </ul>	<ul style="list-style-type: none"> <li>• Refer data sheet for details about incompatible samples</li> <li>• Use the assay buffer provided in the kit or refer data sheet for instructions</li> <li>• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated</li> <li>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope</li> <li>• Aliquot and freeze samples if needed to use multiple times</li> <li>• Troubleshoot if needed, deproteinize samples</li> <li>• Use fresh samples or store at correct temperatures till use</li> </ul>
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> <li>• Improperly thawed components</li> <li>• Use of expired kit or improperly stored reagents</li> <li>• Allowing the reagents to sit for extended times on ice</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw all components completely and mix gently before use</li> <li>• Always check the expiry date and store the components appropriately</li> <li>• Always thaw and prepare fresh reaction mix before use</li> <li>• Refer datasheet &amp; verify correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> </ul>
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> <li>• Use of partially thawed components</li> <li>• Pipetting errors in the standard</li> <li>• Pipetting errors in the reaction mix</li> <li>• Air bubbles formed in well</li> <li>• Standard stock is at an incorrect concentration</li> <li>• Calculation errors</li> <li>• Substituting reagents from older kits/ lots</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw and resuspend all components before preparing the reaction mix</li> <li>• Avoid pipetting small volumes</li> <li>• Prepare a master reaction mix whenever possible</li> <li>• Pipette gently against the wall of the tubes</li> <li>• Always refer the dilutions in the data sheet</li> <li>• Recheck calculations after referring the data sheet</li> <li>• Use fresh components from the same kit</li> </ul>
Unanticipated results	<ul style="list-style-type: none"> <li>• Measured at incorrect wavelength</li> <li>• Samples contain interfering substances</li> <li>• Use of incompatible sample type</li> <li>• Sample readings above/below the linear range</li> </ul>	<ul style="list-style-type: none"> <li>• Check the equipment and the filter setting</li> <li>• Troubleshoot if it interferes with the kit</li> <li>• Refer data sheet to check if sample is compatible with the kit or optimization is needed</li> <li>• Concentrate/ Dilute sample so as to be in the linear range</li> </ul>

**Note:** The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.