Iron Colorimetric 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 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 (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 (Cu^{2+}) interference. The kit measures iron in the linear range of 0.4 to 10 nmole or 8 µM to 200 µM 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°C, protect from light. Warm Assay Buffer to room temperature (RT) 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. Iron Standard Curve:

Dilute 10 µl of the 100 mM Iron Standard with 990 µl dH_20 to generate 1 mM Iron Standard. 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 nmole/well Iron Standard. Add 5 µl Iron Reducer to each Standard well and bring the volume of all Standard wells to 100 µl with Iron Assay Buffer.

2. Sample Test:

Samples can be tested for ferrous (Fe^{2+}), or total (Fe^{2+} and Fe^{3+}) or ferric (Fe^{3+}) ion. Liquid samples such as serum can be tested directly (up to 50 µl of serum per well). Normal serum Iron levels are ~10-40 µM. Soft tissues or cells may be homogenized in 5-10 volumes of Iron Assay Buffer (*i.e.* 500 µl Iron Assay Buffer for each 100 mg of wet tissue or ~5 x 10^6 cells). Thoroughly homogenize tissue samples with a probe sonicator or Dounce glass-bead homogenizer. , then centrifuge at 16000 x *g* for 10 min and transfer the supernatant to a fresh microfuge tube. We suggest testing several doses of your samples to make sure the readings are within the Standard Curve range.

For ferrous Iron (II) assay: Add 1-50 µl sample to sample wells in a 96-well plate and bring the volume to 100 µl/well with Iron Assay Buffer.

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

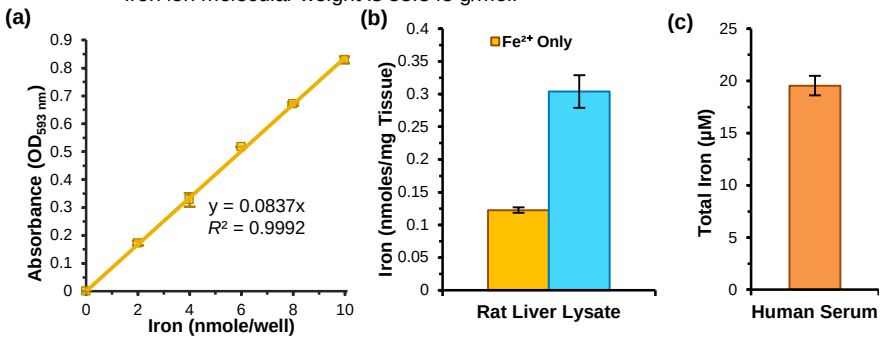
3. Incubate Iron Standards and samples for 30 min at 37 °C.
4. Add 100 µl Iron Probe to each well containing the Iron Standards and test samples. Mix well. Incubate the reaction for 60 min at 37 °C, protected from light.
5. Measure the absorbance at 593 nm ($OD_{593\ nm}$) in a microplate reader.
6. **Calculation:** Subtract the 0 nmole Iron 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:

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

Where, S_a is the iron (II), iron (III), or total iron (II+III) content of unknown samples (in nmoles) from the Standard Curve.

S_v is the sample volume (µl) added into the assay wells.

Iron ion molecular weight is 55.845 g/mol.



Figures: (a) Iron Standard Curve. **(b)**. Assay of iron(II) and total iron(II+III) in perfused rat liver homogenate (an equivalent of 8 mg wet tissue was used per well). **(c)** Assay of total iron(II + III) in off-the-clot human serum (50 µl of serum was added per well). Data are mean ± SEM from 2 independent replicates, performed in duplicate wells.

V. RELATED PRODUCTS:

NAD(P)/NAD(P)H Quantification Kit	ADP/ATP Ratio Assay Kit
Ascorbic Acid Quantification Kit	Glutathione Detection Kit
Total Antioxidant Capacity (TAC) Assay Kit	Fatty Acid Assay Kit
Ethanol Assay Kit	Uric Acid Assay Kit
Pyruvate Assay Kit	Lactate Assay Kit I & II
Ammonia Assay Kit	Free Glycerol Assay Kit
Triglyceride Assay Kit	Hemin Assay Kit
Choline/Acetylcholine Quantification Kit	Glucose Assay Kit
Sarcosine Assay Kit	L-Amino Acid Assay Kit
Glycogen Assay Kit	Cholesterol Assay Kit
Creatinine Assay Kit	HDL & LDL Assay Kit
Creatine Assay Kit	Fatty Acid Assay Kit
Urea Assay Kit	Ammonia Assay Kit

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

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample (e.g. metal ion chelators)	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		