Colorimetric Iron Quantification Assay

Natalie Rubio¹

¹Tufts University

Cellular Agriculture

Natalie Rubio
Tufts University

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.x2ufqew

External link:
[https://www.abcam.com/ps/products/83/ab83366/documents/ab83366%20Iron%20Assay%20Kit%20Protocol%20v11a%20\(web%20site\).pdf](https://www.abcam.com/ps/products/83/ab83366/documents/ab83366%20Iron%20Assay%20Kit%20Protocol%20v11a%20(web%20site).pdf)

Protocol Citation: Natalie Rubio 2019. Colorimetric Iron Quantification Assay.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.x2ufqew>

MANUSCRIPT CITATION:

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol in our group and it is working.

Created: Feb 12, 2019

Last Modified: Feb 12, 2019

ABSTRACT

Measures iron from 0.4 - 20 nmol/83366) to quantify the levels of iron in cell culture and meat samples. Iron dissociates from its carrier protein in the presence of acidic buffer. When reduced to Fe²⁺, ions will react with Ferene S to produce a complex with absorbance at 593 nm. Cu²⁺ interference is blocked with a chelate chemical included in the buffer. This protocol is designed to measure iron from 0.4 - 20 nmol/50 µL sample or 8 µM - 400 µM.

ATTACHMENTS

[Iron Assay Kit Protocol.pdf](#)

GUIDELINES

- Store the kit at -20°C in the dark.
- Do not substitute reagents from other kits.
- Avoid bubbles.
- Avoid cross contamination.

MATERIALS

MATERIALS

- ⊗ Iron Assay Buffer **Abcam Catalog #ab83366**
- ⊗ Iron Probe **Abcam Catalog #ab83366**
- ⊗ Iron Reducer **Abcam Catalog #ab83366**
- ⊗ Iron Standard **Abcam Catalog #ab83366**
- ⊗ UltraPure Distilled Water **Thermo Fisher Scientific Catalog #10977015**
- ⊗ Dulbeccos Phosphate Buffered Saline **Thermo Fisher Scientific Catalog #14040133**

SAFETY WARNINGS

- Reagents should be treated as possible mutagens and disposed of properly.

BEFORE START INSTRUCTIONS

- Ensure you have sufficient reagent amounts to perform the assay for the number of samples you intend to run.

Prepare the reagents from the Iron Assay Kit.

1. Equilibrate the iron standard, iron assay buffer and iron reducer to room temperature.
2. Thaw the iron probe and then keep on ice during the duration of the assay. 01:00:00

Prepare the standard curve.

1. Dilute 10 μL of iron standard in 990 μL of distilled water to prepare a 1 mM standard. Prepare a standard curve dilution in a 96-well plate.

	Standard No.	1 mM Iron Standard Volume (μL)	Assay Buffer Volume (μL)	Final Volume (μL)	Iron Concentration (nmol/well)
	1	0	300	100	0
	2	6	294	100	2
	3	12	288	100	4
	4	18	282	100	6
	5	24	276	100	8
	6	30	270	100	10

Standard curve dilution.

2. Set up the dilution for a duplicate reading (2 x 100 μL).

Prepare the samples.

- 3**
1. Use fresh samples or thaw snap frozen samples on ice. ⌚ 02:00:00
 2. Weigh 10 mg of tissue sample for each condition.
 3. Wash the tissue in cold PBS.
 4. Homogenize each tissue in 300 μ L iron assay buffer with a Dounce homogenizer on ice with 15 passes.
 5. Transfer the homogenates from the homogenizer to microcentrifuge tubes.
 6. Centrifuge the homogenates at 16,000 g for 10 minutes to remove insoluble particles. ⌚ 00:10:00
 7. Transfer the supernatants to clean microcentrifuge tubes and keep on ice.

Perform the assay.

- 4**
1. Add 100 μ L of standard dilutions in duplicate to a 96-well plate.
 2. Add 50 μ L of each sample in quadruplicate (duplicate for Fe^{2+} , duplicate for total Fe) to the 96-well plate and add 50 μ L iron assay buffer to each sample to bring the total volume to 100 μ L per well.
 3. Add 5 μ L of iron reducer to each standard well.
 4. For Fe^{2+} measurement, add 5 μ L of assay buffer to each sample.
 5. For total Fe measurement, add 5 μ L of iron reducer to each sample.
 6. Mix and incubate standards and samples at 25°C for 30 minutes. ⌚ 00:30:00
 7. Add 100 μ L iron probe to each well containing the iron standard and test samples.
 8. Mix and incubate at 25°C for 60 minutes, in the dark. ⌚ 01:00:00
 9. Measure output on a colorimetric microplate reader at OD 593.

Calculate iron concentrations.

- 5**
1. Average the duplicate reader for each standard and sample.
 2. Subtract the mean absorbance value of the blank (standard no. 1) from all standard and sample readings to obtain the corrected absorbance.
 3. Plot the corrected absorbance values for each standard as a function of the final iron concentration.
 4. Calculate the line of best fit.

Expected result

$$y = 0.0826x + 0.0199, R^2 = 0.9987$$

5. Determine Fe^{2+} and total Fe from the standard curve.
6. Calculate Fe^{3+} by subtracting Fe^{2+} from the total Fe.
7. Iron concentration is calculated as: $[C] = (\text{Sa}/\text{Sv}) * D$
 - [C] = iron concentration
 - Sa = content of iron in well calculated from standard curve (nmol)
 - Sv = volume of sample added into the reaction wells (μL)
 - D = sample dilution factor (if necessary)
 - (The molecular weight of iron is 55.845 g/mol.)