Colorimetric Iron Quantification Assay

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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 Fe^{2+}, ions will react with Ferene S to produce a complex with absorbance at 593 nm. Cu^{2+} 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.

EXTERNAL LINK

https://www.abcam.com/ps/products/83/ab83366/documents/ab83366%20Iron%20Assay%20Kit%20Protocol%20v11a%20(website).pdf

PDF

Iron Assay Kit Protocol.pdf

PROTOCOL STATUS

Working

We use this protocol in our group and it is working.

GUIDELINES

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

MATERIALS

NAME	CATALOG #	VENDOR
Iron Assay Buffer	ab83366	Abcam
Iron Probe	ab83366	Abcam
Iron Reducer	ab83366	Abcam
Iron Standard	ab83366	Abcam
UltraPure Distilled Water	10977015	Thermo Fisher Scientific
Dulbeccos Phosphate Buffered Saline	14040133	Thermo Fisher Scientific

SAFETY WARNINGS

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

BEFORE STARTING

- 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

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.

2

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 µL 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 *xg* 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 µL of standard dilutions in duplicate to a 96-well plate.
2. Add 50 µL of each sample in quadruplicate (duplicate for Fe^{2+}, duplicate for total Fe) to the 96-well plate and add 50 µL iron assay buffer to each sample to bring the total volume to 100 µL per well.
3. Add 5 µL of iron reducer to each standard well.
4. For Fe^{2+} measurement, add 5 µL of assay buffer to each sample.
5. For total Fe measurement, add 5 µL 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 µL 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. Substrate 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: $[C] = (Sa/Sv) * D$

[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.)