

Amplite® Colorimetric Iron Assay Kit

 Catalog number: 19008, 19009
 Unit size: 100 Tests, 500 Tests

Component	Storage	Amount (Cat No. 19008)	Amount (Cat No. 19009)
Component A: FerroBrite™ Purple 590	Freeze (< -15 °C), Minimize light exposure	1 Vial	5 Vials
Component B: Iron Reducer	Freeze (< -15 °C), Minimize light exposure	1 Vial	5 Vials
Component C: Assay Buffer	Freeze (< -15 °C), Minimize light exposure	1 Bottle (25 mL)	1 Bottle (125 mL)
Component D: Iron Standard	Freeze (< -15 °C)	1 Vial (100 µL)	1 Vial (100 µL)

OVERVIEW

The Amplite® Colorimetric Iron Assay Kit provides a simple and reliable method for quantifying total iron in biological and environmental samples. The assay is based on FerroBrite™ Purple 590, a chromogenic reagent that reacts specifically with ferrous iron (Fe²⁺) to form a purple-colored complex. To enable measurement of total iron, ferric iron (Fe³⁺) present in the sample is first reduced to Fe²⁺. The resulting color intensity, measured at 590 nm, is directly proportional to the iron concentration in the sample, with a linear detection range of 8 µM to 200 µM.

Iron measurement is an important parameter in both research and clinical applications, as abnormal levels can reflect a range of physiological or pathological conditions. Elevated iron concentrations may occur in cases of red blood cell destruction, decreased blood cell survival, acute hepatitis, sideroblastic anemias, high dietary iron intake, or impaired iron storage. Decreased iron levels are often associated with insufficient dietary intake, chronic blood loss, or increased demand during pregnancy. The Amplite® kit is well-suited for measuring iron in biological fluids such as plasma and serum (without pretreatment) and in environmental samples such as soil extracts, and for investigating the effects of drugs or other interventions on iron metabolism. The method offers sensitivity, reproducibility, and a straightforward workflow, making it suitable for studies of iron homeostasis in health and disease.

AT A GLANCE
Protocol Summary

1. Prepare test samples and iron standards (100 µL).
2. Add iron reducer (10 µL).
3. Incubate at room temperature for 5 minutes.
4. Add FerroBrite™ Purple 590 assay working solution (100 µL).
5. Measure absorbance at 593 nm.

Important Note

Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS
Absorbance microplate reader

Absorbance	593 nm
Recommended plate	Clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Iron Reducer

1. Prepare the Iron Reducer stock solution by adding 1 mL of water to the vial of Iron Reducer (Component B).

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
<https://www.aatbio.com/tools/serial-dilution/19008>

Iron Standard Dilution

To prepare the iron standards, first add 2 µL of Iron Standard Solution (Component D) to 1 mL of Assay Buffer (Component C) to obtain a 200 µM solution, designated as STD7. From this stock, perform a series of twofold serial dilutions in Assay Buffer to generate standards STD6 through STD1. Specifically, transfer 500 µL of STD7 into 500 µL of Assay Buffer to prepare STD6, then continue this process sequentially by mixing 500 µL of each preceding dilution with 500 µL of Assay Buffer until STD1 is obtained.

PREPARATION OF WORKING SOLUTION
FerroBrite™ Purple 590 Working Solution

1. To prepare the FerroBrite™ Purple 590 working solution, add 10 mL of Assay Buffer (Component C) to the FerroBrite™ Purple 590 vial (Component A) and mix until fully dissolved.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of iron standards, and test samples in a clear-bottom 96-well microplate. STD = Iron Standards (STD1-STD7, 3.1 to 200 µM), BL = Blank Control, TS = Test Samples.

	BL	BL	TS	TS
STD 1	STD 1	
STD 2	STD 2	
STD 3	STD 3			
STD 4	STD 4			
STD 5	STD 5			
STD 6	STD 6			
STD 7	STD 7			

Table 2. Reagent composition for each well.

Well	Volume	Reagent
STD 1 - STD 7	100 µL	Serial Dilutions (3.1 to 200 µM)
BL	100 µL	Assay Buffer
TS	100 µL	Test Sample

1. Prepare iron standards (STD1-STD7), blank controls (BL), and test samples (TS) following the plate layout provided in Tables 1 and 2.

When using a 384-well plate, dispense 50 μL of reagent per well.
For all other plate formats, dispense 100 μL per well.

2. For the detection of ferrous iron (Fe^{2+}), add 10 μL of assay buffer to each test sample well.
3. For total iron ($\text{Fe}^{2+} + \text{Fe}^{3+}$) determination, add 10 μL of the iron reducer stock solution to each test sample well.
4. Incubate at room temperature for 5 minutes.
5. Add 100 μL of FerroBrite™ Purple 590 working solution to each well containing the blank control, iron standards, and test samples.
6. Monitor the absorbance increase with an absorbance plate reader at 593 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

Absorbance values from blank wells are used as background controls and subtracted from the readings of both iron standards and test samples. The resulting values are applied to the standard curve generated from the iron standards (Figure 1) to determine sample iron concentrations. For accuracy and ease of calculation, we recommend using the Online Linear Regression Calculator, which can be found at:

<https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator>

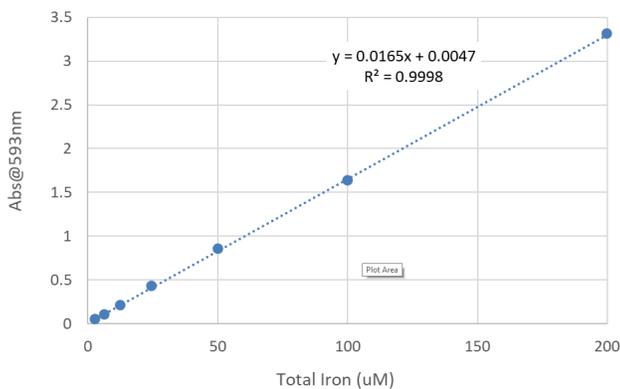


Figure 1. The iron standard curve was generated using the Amplitude® Colorimetric Iron Assay Kit in a 96-well clear-bottom microplate, and absorbance was measured at 593 nm with a ClarioStar microplate reader (BMG).

DISCLAIMER

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