ScienCell™
Research Laboratories

Iron Assay
(Ir)
Cat. No. 8448
100 Tests in 96-well plate

Introduction

Iron is an element that plays an essential role in a number of biological processes. Iron is bound to several proteins such as the oxygen-transporting hemoglobin in the red blood cells and oxygen storage protein myoglobin in the muscle. It is also stored by ferritin and hemosiderin. Inorganic iron contributes to redox reactions in the iron-sulfur clusters of many enzymes, such as nitrogenase. In this colorimetric assay, ferric carrier protein will dissociate ferric into solution under acidic conditions. After they are reduced to the ferrous ions, these ions react with chromogen to produce a stable colored complex and give absorbance at 590 nm. The color intensity thus formed is directly proportional to the concentration of iron in the sample. A specific chelate chemical is included in the buffer to block copper ion interference.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8448a	1	Assay buffer	30 mL	4°C
8448b	1	Iron standard	0.2 mL	-20°C
8448c	1	Dilution buffer	10 mL	RT*
8448d	1	Sample reagent	1.5 mL	RT*
8448e	1	Reductant	1.5 mL	4°C
8448f	1	Chromogen	1 mL	-20°C

* RT denote room temperature

Product Use

The Iron Assay kit measures the total iron level of different types of samples, such as cell lysate, tissue extracts, serum and plasma. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Data from the Iron Assay kit measuring iron solutions with concentrations ranging from 6.25 to 200 µM show a linear relationship between OD_{590nm} and iron concentration (Figure 1). Linear detection range is 1.56 to 500 µM in 96-well plate assay.

Shipping

Shipped on dry ice.

Procedure (96-well plate)

A. Preparation of iron standard

1. Add 1 µL of iron standard (8448b) to 499 µL of dilution buffer (8448c) to make a 400 µM iron standard.
2. Obtain 7 test tubes, add 120 µL of dilution buffer (8448c) into each tube and label them #1 through #7.
3. Add 120 µL 400 µM iron standard into tube #1 and mix well to get the 200 µM iron standard.
4. Transfer 120 µL of the 200 µM iron standard from tube #1 to tube #2 and mix well to get the 100 µM iron standard.
5. Repeat step 4 for tubes #3-6 to serially dilute the iron standards. Do not add any iron to tube #7, which serves as the blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each iron standard by aliquoting 50 µL/well of each iron standard into duplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7
A	200µM	100µM	50µM	25µM	12.5µM	6.25µM	Blank
B	200µM	100µM	50µM	25µM	12.5µM	6.25µM	Blank

B. Preparation of test samples

1. Cells or tissues can be homogenized in 4 volumes of the assay buffer (8448a). Centrifuge the samples at 13,000 ×g for 10 minutes to remove insoluble material.
2. Mix sample (cell lysate or serum) with equal volume solution of 93 volumes of assay buffer (8448a), 2 volumes reductant (8448e) and 5 volumes sample reagents (8448d).
3. Centrifuge the mixed sample 13,000 ×g for 5 minutes and take the supernatant as the test samples for the iron assay.
4. Test samples should be serial diluted to make sure the readings are within the standard curve range. Prepare test samples to a final volume of 50 µL/well on the 96-well flat bottom plate.

C. Working reagent preparation and measurements

1. For each well of reaction, prepare working reagent by mixing 200 µL assay buffer (8448a), 10 µL reductant (8448e) and 10 µL chromogen (8448f).
2. Add 200 µL of working reagent mix into each well of the 96-well plate containing iron standard, test samples and blank. Incubate for 30 minutes at room temperature in dark.
3. Read the absorbance at 590 nm with an ELISA plate reader.

D. Calculations

1. Subtract the OD_{590nm} value of the blank from the OD_{590nm} values obtained with all other standard and samples to get ΔOD_{590nm} value.
2. Based on the calibrated OD_{590nm} of the iron standard, make a standard curve by plotting ΔOD_{590nm} as a function of iron concentration (See Figure 1 for a typical standard curve). Determine the equation and R^2 value of the trend line.
3. Supposing the equation of the trend line of the standard curve is $y = Ax + B$, calculate the iron concentration of test samples as follows:

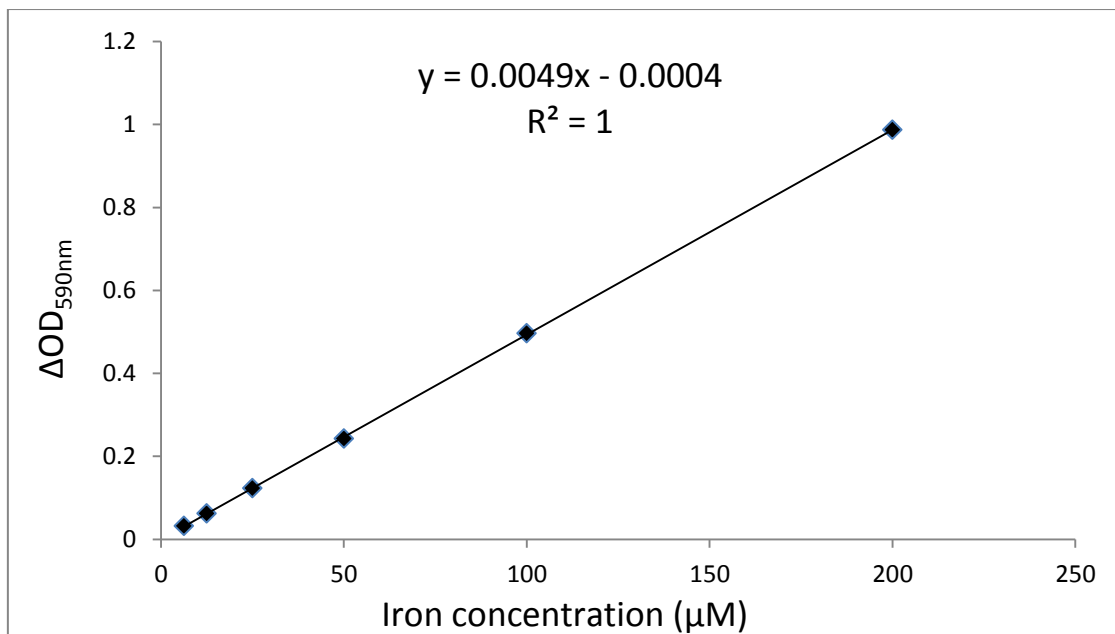$$[Iron] = 2 \times \frac{\Delta OD_{590nm} - B}{A}$$

Figure1. A typical iron standard curve measured by the ScienCell™ Iron Assay kit