Signosis, Inc.
Innovative Plate Assay Solutions

Ferrozine Iron Assay Kit

Catalog # EA-7101 **(For Research Use Only)**

Introduction

The Ferrozine Iron Assay Kit utilizes ferrozine to detect ferrous iron (Fe^{2+}) in in cells, tissues, or biological fluids. Iron is an essential trace element involved in oxygen transport, energy metabolism, DNA synthesis, and redox regulation. Dysregulation of cellular iron homeostasis has been implicated in oxidative stress, ferroptosis, neurodegenerative disorders, cardiovascular disease, and cancer.

In this assay, ferrous iron reacts with ferrozine to form a purple-colored complex that can be quantified spectrophotometrically at an absorbance of 562 nm. Ferric iron (Fe^{3+}) can also be measured following reduction to Fe^{2+}, allowing determination of total iron content. The intensity of the colored complex is directly proportional to the iron concentration in the sample, providing a simple, sensitive, and reliable method for quantifying iron in cell lysates, tissue homogenates, serum, plasma, and other biological samples.

Materials Required but Not Provided

- 96-well clear microplate
- Microplate reader capable of measuring absorbance at 562nm

Materials Provided

- Iron (Fe^{2+}) Standard (-20°C)
- Reduction Reagent (-20°C)
- 10mM Ferrozine Stock (-20°C)

****Spin down small tubes before starting experiment. ****

Plasma Sample Preparation

1. Centrifuge citrated or EDTA-collected blood at 4°C (1,000 x *g* for 10 minutes) to separate plasma from erythrocytes. Alternatively, blood collected without anticoagulant can be centrifuged to collect serum.
2. Transfer the plasma layer to a new tube without disturbing the buffy layer.
3. The plasma may be assayed directly or stored away at -80°C.

Cell Sample Preparation

1. Wash the cells once with PBS before lysing the cells.
2. For a 96-well culture plate, add 40 µL of lysis buffer to each well and incubate at room temperature for 10 minutes.
3. Pipette the lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
4. If necessary, homogenize the cell lysates with a sonicator.
5. The cell lysates may be assayed directly or stored at -80°C.
6. Use PBS to dilute the cell sample to the appropriate concentration for each assay, if necessary.

Tissue Sample Preparation

1. Weigh tissue sample and add 1 mL of lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C.
6. Use PBS to dilute the tissue sample to the appropriate concentration for each assay, if necessary.

Iron Measurement

1. Using the provided 1 mM iron (Fe^{2+}) standard, prepare a standard curve dilution in a 96-well clear plate as described in the table below:

Standard #	1	2	3	4	5	6
Iron Standard Volume (µL)	5	4	3	2	1	0
ddH2O (µL)	45	46	47	48	49	50
Iron Final Concentration (µM)	100	80	60	40	20	0
Standard Final Volume (µL)	50	50	50	50	50	50

Iron Standard Dilution Table

2. Prepare samples in each well of the plate by diluting 10 µL of the sample in 40 µL of ddH2O.
3. For total iron measurement (Fe^{2+} and Fe^{3+}), add 5 µL of the reduction reagent to each sample or standard in the 96-well plate.
4. Mix and incubate the standards and samples at 37°C for 30 minutes.
5. Make detection solution by diluting the 10mM Ferrozine stock 1:10 in ddH2O.
6. Add 50 µL of detection solution to each well containing standards or samples and incubate at room temperature for 5-10 minutes away from light.
7. Measure the absorbance of the plate at 562 nm using a plate reader.