

Ferric-Chelate Reductase (FCR) Activity Assay Kit - Microplate Method**Product Code:** F930372**Product Introduction**

Ferric-chelate reductase (FCR) catalyzes the reduction of ferric chelates from Fe^{3+} to Fe^{2+} and plays an important role in iron uptake in some species.

In this assay, FCR reduces Fe^{3+} to Fe^{2+} . The Fe^{2+} then reacts with ferrozine to produce color with a characteristic absorbance at 562 nm.

Package Contents

Pack Size	Item	Contents
100T	BR5000839.1	Reagent I, 1 bottle
100T	BR5000839.2	Reagent II, 1 bottle
100T	BR5000839.3	Reagent III, 1 bottle
100T	BR5000839.m	Instructions, 1 copy

Quality Standards and Safety

Raw Material and Packaging Name	Quality Standard	Main Toxicity
Reagent I	—	—
Reagent II	—	—
Reagent III	—	—

Transportation and Storage**Transportation:** Transport with ice packs.**Storage:** Store at 2-8°C, protected from light. Shelf life: 180 days.**Required Instruments and Supplies**

Microplate reader, benchtop centrifuge, adjustable pipette, 96-well plate, mortar, ice, and distilled water.

Instructions for Use**1. Preparation of Crude Enzyme Extract**

1. Prepare the extract using a tissue mass (g) to water (mL) ratio of 1:5-10.
2. It is recommended to weigh about 0.1 g of tissue and add 1 mL of distilled water.
3. Homogenize in an ice bath.
4. Centrifuge at 10000 g, 4°C for 10 min.
5. Collect the supernatant and keep it on ice for testing.

2. Assay Procedure

1. Preheat the microplate reader for more than 30 min, set the wavelength to 562 nm, and zero with distilled water.

2. Prepare the working solution by mixing Reagents I, II, and III at a 1:1:1 ratio. Prepare immediately before use and only in the amount needed.
3. In a 96-well plate, add 50 μL of sample supernatant and 150 μL of working solution.
4. Mix well and record the initial absorbance value A_1 .
5. Record the absorbance again after 30 min as A_2 .
6. Calculate $\Delta A = A_2 - A_1$.

Activity Calculation

Using a 96-well plate, the standard curve is:

$$y = 4.0007x + 0.0011, R^2 = 0.9997$$

y: absorbance.

3.1 Calculated by Sample Mass

Unit definition: the amount of enzyme that produces 1 nmol of Fe^{2+} -ferrozine per minute per gram of sample is defined as one enzyme activity unit.

$$\text{FCR (nmol/min/g fresh weight)} = (\Delta A - 0.0011) \div 4.0007 \times 1000 \times V_{\text{std}} \div (V_{\text{sample}} \div V_{\text{total sample}} \times W) \div T = 8.331 \times (\Delta A - 0.0011) \div W$$

3.2 Calculated by Sample Protein Concentration

Unit definition: the amount of enzyme that produces 1 nmol Fe^{2+} -ferrozine per minute per mg protein is defined as one enzyme activity unit.

$$\text{FCR (nmol/min/mg prot)} = (\Delta A - 0.0011) \div 4.0007 \times 1000 \times V_{\text{std}} \div (V_{\text{sample}} \div V_{\text{total sample}} \times C_{\text{pr}}) \div T = 8.331 \times (\Delta A - 0.0011) \div C_{\text{pr}}$$

Parameter Definitions

Parameter	Definition
$V_{\text{total sample}}$	Volume of extraction solution added, 1 mL
V_{sample}	Reaction sample volume, 50 μL
V_{std}	Volume of standard added, 50 μL
T	Reaction time, 30 min
W	Sample weight, g
C_{pr}	Sample protein concentration, mg/mL
1000	μmol to nmol conversion factor

Precautions

Before the formal assay, select 2-3 samples with large expected differences for pretesting.