Ferric-Chelate Reductasee(FCR) Activity Assay Kit

Product Code: 112914

Ferric-Chelate Reductase (FCR) Activity Assay Kit - Micro Method

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Product Introduction

Ferric-chelate reductase (FCR) catalyzes the reduction of ferric chelates, converting $Fe^{3+}$to Fe^{2+}. This enzyme plays an important role in iron absorption 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 Code	Description	Quantity
100T	112914.1	Reagent I	1 bottle
100T	112914.2	Reagent II	1 bottle
100T	112914.3	Reagent III	1 bottle
100T	112914.m	Manual	1 copy

Quality Standards and Safety Information

Raw Materials 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.

Instructions for Use

1. Preparation of Crude Enzyme Extract

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

2. Assay Procedure

1. Preheat the spectrophotometer or microplate reader for more than 30 min, set the wavelength to 562 nm, and zero with distilled water.
2. Prepare the working solution fresh before use by mixing Reagents I, II, and III at a 1:1:1 ratio. Prepare only the amount needed.

3. In a micro quartz cuvette or 96-well plate, add 50 μL sample supernatant and 150 μL working solution.
4. Mix well and record the initial absorbance as A1 and the absorbance after 30 min as A2.
5. Calculate ΔA = A2 - A1.

Activity Calculation

3.1 Micro Quartz Cuvette Method

Standard curve: y = 8.0014x + 0.0011, R^2= 0.9997

3.1.1 Calculated by Sample Mass

Unit definition: One unit of enzyme activity is defined as the production of 1 nmol Fe^{2+}-ferrozine per minute per g of sample.

FCR (nmol/min/g fresh weight) = (ΔA - 0.0011) ÷ 8.0014 × 1000 × V_{std}÷ (V_{sample}÷ $V_{total\ sample}$× W) ÷ T = 4.166 × (ΔA - 0.0011) ÷ W

3.1.2 Calculated by Sample Protein Concentration

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

FCR (nmol/min/mg prot) = (ΔA - 0.0011) ÷ 8.0014 × 1000 × V_{std}÷ (V_{sample}÷ $V_{total\ sample}$× Cpr) ÷ T = 4.166 × (ΔA - 0.0011) ÷ Cpr

Where:

- $V_{total\ sample}$: volume of extraction solution added, 1 mL
- V_{sample}: sample volume in the reaction, 50 μL
- V_{std}: volume of standard added, 50 μL
- T: reaction time, 30 min
- W: sample mass, g
- Cpr: sample protein concentration, mg/mL
- 1000: conversion factor from μmol to nmol

3.2 96-Well Plate Method

Standard curve: y = 4.0007x + 0.0011, R^2= 0.9997

y: absorbance

3.2.1 Calculated by Sample Mass

Unit definition: One unit of enzyme activity is defined as the production of 1 nmol Fe^{2+}-ferrozine per minute per g of sample.

FCR (nmol/min/g fresh weight) = (ΔA - 0.0011) ÷ 4.0007 × 1000 × V_{std}÷ (V_{sample}÷ $V_{total\ sample}$× W) ÷ T = 8.331 × (ΔA - 0.0011) ÷ W

3.2.2 Calculated by Sample Protein Concentration

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

FCR (nmol/min/mg prot) = (ΔA - 0.0011) ÷ 4.0007 × 1000 × V_{std}÷ (V_{sample}÷ $V_{total\ sample}$× Cpr) ÷ T = 8.331 × (ΔA - 0.0011) ÷ Cpr

Where:

- $V_{total\ sample}$: volume of extraction solution added, 1 mL
- V_{sample}: sample volume in the reaction, 50 μL
- V_{std}: volume of standard added, 50 μL
- T: reaction time, 30 min
- W: sample mass, g
- Cpr: sample protein concentration, mg/mL

- 1000: conversion factor from μmol to nmol

Precautions

> Before the formal assay, select 2-3 samples with large expected differences for pretesting.
>
> Required instruments and supplies: visible spectrophotometer or microplate reader, benchtop centrifuge, adjustable pipette, micro quartz cuvette or 96-well plate, mortar, ice, and distilled water.