CheKine™ Micro Ferrate reductase (FCR) Activity Assay Kit

Cat #: KTB1621 Size: 48 T/48 S 96 T/96 S

	Micro Ferrate reductase (FCR) Activity Assay Kit		
REF	**Cat #:** KTB1621	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Ferric reductase (FCR) catalyzes the reduction of Fe^{3+} to Fe^{2+}, in high valent iron chelates, which plays an important role in the absorption of iron in some species. Fe^{2+} will react with ferrozine to develop color, and there is a characteristic absorption value at 562 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	65 mL	65×2 mL	4℃, protected from light
Reagent Ⅰ	3 mL	6 mL	4℃, protected from light
Reagent Ⅱ	3 mL	6 mL	4℃
Standard	Powder×1 vial	Powder×1 vial	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 562 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic water bath, ice maker, centrifuge, incubator
- Deionized water, sulfuric acid
- Mortar or homogenizer

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent: Prepared before use. According to the experimental dosage, Extraction Buffer, Reagent I, Reagent II will be mixed at 1:1:1, now used.

Standard: Prepared before use. 50 umol/mLFe^{2+} standard solution was prepared by adding 0.71 mL deionized water and 10 μL sulfuric acid, and the prepared standard solution can be stored at 4℃ for 2 weeks.

Note: Standard has low toxicity, so it is recommended to experiment in a fume hood.

50 nmol/mL standard solution: Prepare and use immediately. Before use, 20 μL 50 μmol/mL Fe^{2+} standard solution was mixed with 980 μL deionized water to prepare 1 μmol/mL standard solution, and then 50 μL 1 μmol/mL (1,000 nmol/mL) and 950 μL deionized water were mixed to prepare 50 nmol/mL standard solution.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissue samples: Weigh about 0.1 g tissue samples, add 1 mL ExtractionBuffer, ice bath homogenate, centrifuge 10 min at 15,000g at 4℃, take the supernatant and put it on ice to be tested.
2. Serum (plasma) and other liquid samples: Direct determination. If the liquid is turbid, the supernatant is determined by centrifugation.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 562 nm, visible spectrophotometer was returned to zero with deionized water.
2. Blank well determination: 50 μL deionized water and 150 μL Working Reagent were added to the microglass cuvette or 96-well plate, and the absorbance was recorded A_{Blank}. The blank well only needs to be done 1-2 times.
3. Standard well determination: add 50 μL 50nmol/mL Fe^{2+} standard solution and 150 μL Working Reagent were added to the microglass cuvette or 96-well plate, mix well, record the absorbance $A_{Standard}$, calculate $\Delta A_{Standard}=A_{Standard}-A_{Blank}$, then the final concentration of Fe^{2+} is 12.25 nmol/mL, and the standard well only needs to be done 1-2 times.
4. Sample determination: 50 μL sample supernatant and 150 μL Working Reagent were added to a microglass cuvette or 96-well plate, and the initial absorbance A_1 and room temperature 30 min absorbance A_2 were recorded. Calculated $\Delta A_{Test}=A_2-A_1$.

Note: Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If the ΔA_{Test} is less than 0.01, the sample size can be increased or the reaction time can be prolonged before the determination; if the ΔA_{Test} is greater than 1.5, it is recommended that the supernatant of the sample should be diluted with Extraction Buffer before determination. Pay attention to the synchronous modification of the calculation formula.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculated by protein concentration

Active unit definition: The production of 1 nmol Fe^{2+}-ferrozine per min per mg tissue protein is defined as a unit of enzyme activity.

FCR (U/mg prot)=$\Delta A_{test}\times C_{standard}\div\Delta A_{standard}\times V_{Total}\div(V_{Sample}\times Cpr)\div T$**=$1.67\times\Delta A_{test}\div\Delta A_{standard}\div Cpr$**

2. Calculated by sample fresh weight

Active unit definition: The production of 1 nmol Fe^{2+}-ferrozine per min per g tissue is defined as a unit of enzyme activity.

FCR (U/g fresh weight)=$\Delta A_{test}\times C_{standard}\div\Delta A_{standard}\times V_{Total}\div(W\times V_{Sample}\div V_{Total\ Sample})\div T$**=$1.67\times\Delta A_{test}\div\Delta A_{standard}\div W$**

3. Calculated by sample volume

Active unit definition: The production of 1 nmol Fe^{2+}-ferrozine per min per mL serum (plasma) is defined as a unit of enzyme activity.

FCR (U/mL)=$\Delta A_{test} \times C_{standard} \div \Delta A_{standard} \times V_{Total} \div V_{Sample} \div T$=**$1.67 \times \Delta A_{test} \div \Delta A_{standard}$**

$C_{standard}$: final concentration of Fe^{2+} standard solution 12.5 nmol/mL; V_{Total}: total reaction volume, 0.2 mL; V_{Sample}: sample volume added, 0.05 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; W: sample mass, g; T: reaction time, 30 min.

Typical Data

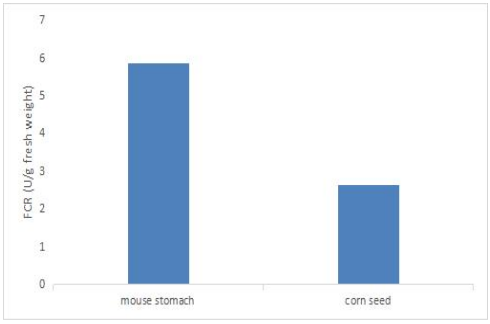

Figure 1. Determination FCR activity in mouse stomach and corn seed by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.