Ferric chelate reductase Activity Assay Kit

Detection Equipment: Spectrophotometer/microplate reader

Catalog Number: BC5915

Size: 100T/96S

Components:Please carefully check the volume of the reagent and the volume in the bottle before use. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation condition
Extract	Liquid110 mL×1	2-8℃ storage
Reagent I	Liquid 6 mL×1	2-8℃ storage
Reagent II	Liquid 10 mL×1	2-8℃ storage
Reagent III	Liquid 6 mL×1	2-8℃ storage
Standard	Powder ×1	2-8℃ storage

Solution reparation :

1. Color development solution: According to the sample quantity before use, according to the ratio of Reagent I: Reagent II: Reagent III =50μL: 50μL: 50μL (150μL, 1T), the color development solution was prepared, mixed thoroughly, and now used;
2. Standard: Add 0.71mL distilled water and 10μL concentrated sulfuric acid to prepare 50μmol/mL Fe^{2+} Standard solution before use. The dissolved Standard can be storage for 2 weeks at 2-8℃.
3. Preparation of 62.5nmol/mL Standard solution: 2.5μmol/mL Standard solution was prepared by mixing 50μL of 50μmol/mL Fe^{2+} Sandard solution and 950μL of distilled water before use. Then 25μL 2.5μmol/mL (2500nmol/mL) and 975μL distilled water were mixed to prepare 62.5nmol/mL Standard solution for later use.

Description:

Dicots and non-gramineous monocots use efficient activation and uptake mechanisms of iron chelation reduction reactions to obtain iron from soil. Ferric iron can be absorbed and utilized by plants only after it is reduced to divalent iron. When Fe(III) -chelate is sufficient, the trivalent Fe oxidoreductases in plant roots reduce Fe from Fe(III) -chelate and transport the reduced Fe^{2+} through the plasma membrane into the root cells.

Ferric chelate reductase (FCR, EC1.16.1.7) catalyzes the reduction of Fe^{3+} to Fe^{2+}. Fe^{2+} and phenoxazine form a purple complex with a characteristic absorption peak at 562nm

$$Fe^{3+} \xrightarrow{FCR} Fe^{2+}$$

$$Fe^{2+} + \text{Ferrozine} \longrightarrow [Fe(\text{Ferrozine})_3]^{2+} \text{（562nm）}$$

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorbance value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, cryogenic centrifuge, analytical balance, micro glass cuvette/96 well flat-bottom plate, adjustable pipetting gun, mortar/homogenizer, concentrated

sulfuric acid (95%-99% AR), ice and distilled water.

Protocol:

I. Sample processing (The sample size to be tested can be appropriately adjusted, and the specific proportion can be referred to the literature)

1. Tissue sample: Add the extract solution according to the ratio of mass (g) : extraction liquid volume (mL) : 1:5-10 (it is recommended to weigh 0.1g sample and add 1.0mL extraction solution). After homogenization in an ice bath, centrifuge at 8000g at 4℃ for 10min, discard the precipitate, and take the supernatant and place it on ice for testing.

2. Liquid sample: Direct measurement. If the liquid was cloudy, the supernatant was centrifuged for determination.

II. Measurement Steps

1. The visible spectrophotometer was preheated for more than 30min, the wavelength was adjusted to 562nm, and zero it with distilled water.
2. The chromogenic solution was equilibrated to room temperature before use.
3. Standard tube determination

   Absorb 50μL of 62.5nmol/mL Fe^{2+} standard solution, add 150μL of color development agent, thoroughly mix, measure the absorbance at 562nm, recorded as standard A, at this time, the final concentration of Fe^{2+} is 15.625nmol/mL, the standard tube only needs to do 1-2 times.
4. Blank tube test

   Absorb 50μL of distilled water, add 150μL of color development agent, mix thoroughly, measure the absorbance at 562nm, recorded as A blank, calculate $\Delta A_{standard} = A_{standard} - A_{blank}$, blank tube only need to do 1-2 times.
5. Operation table: (the following reagents in micro glass cuvette/96 well flat-bottom plate)

Reagent name（μL）	Test tube
Sample	50
Color developing solution	150
Immediately mix thoroughly in micro glass cuvette/96 well flat-bottom plate, the absorbance value A1 at 10s was measured at 562nm, and the reaction time was 30min at room temperature. The absorbance value A2 at 10s was measured at 30min, and the absorbance value A1 at 10s at 562nm and A2 after 30min were recorded. ΔA=A2-A1 was calculated.	

III. Calculations

1. According to the sample protein concentration:

Definition of unit: 1nmol Fe^{2+} produced per minute per mg of tissue protein was defined as one unit of enzyme activity.

FCR activity (U/mg prot) = $\Delta A \times C_{standard} \div \Delta A_{standard} \times V_{total} \div (Cpr \times V_{sample}) \div T = 2.083 \times \Delta A \div \Delta A_{standard} \div Cpr$

2. According to sample quality:

Definition of unit: 1nmol Fe^{2+} produced per minute per g of tissue was defined as one unit of

enzyme activity.

FCR activity (U/g) = ΔA× $C_{standard}$ ÷$ΔA_{standard}$ ×$V_{total\ reaction}$ ÷(W÷$V_{extraction}$ ×V_{sample}) ÷T =2.083×ΔA÷$ΔA_{standard}$ ÷W

$C_{standard}$: Fe^{2+} standard final concentration, 15.625nmol/mL;

$V_{total\ reaction}$: total volume of reaction system, 0.2mL;

Cpr: supernatant protein concentration, mg/mL;

V: Add the volume of supernatant liquid in the reaction system, 50μL=0.05mL;

$V_{extraction}$: add extraction liquid volume, 1mL;

T: reaction time, 30min;

W: sample quality, g.

Note:

1. If ΔA is less than 0.010 or the absorbance value of the measuring tube is close to the blank tube, the sample size can be increased or the reaction time can be prolonged before the measurement; If ΔA is greater than 1 or A1 is greater than 1, it is recommended that the sample supernatant be appropriately diluted with the extract before performing the assay. Note that the calculation formula is modified synchronously.

Experimental example:

1. Weigh 0.1214g of cherry tomatoes, add the extract to the ice bath homogenate, and follow the determination steps. Calculated with 96 well flat-bottom plate ΔA= A2-A1=0.665-0.127=0.538, $ΔA_{standard}$ =$A_{standard}$-A_{blank} =0.525-0.083=0.442, and put them into the formula to calculate:
   FCR activity (U/g mass) =2.083×ΔA÷$ΔA_{standard}$ ÷W=20.885 U/g mass
2. Weigh 0.1002g Minnan leaves, add the extract for ice bath homogenization, and follow the determination steps. Calculated with 96 well flat-bottom plate ΔA= A2-A1=0.188-0.104=0.084, $ΔA_{standard}$ =$A_{standard}$-A_{blank} =0.525-0.083=0.442, and put them into the formula to calculate:
   FCR activity (U/g mass) =2.083×ΔA÷$ΔA_{standard}$ ÷W=3.951U/g mass

References:

[1] Shabnam N , Kim M , Kim H .Iron (III) oxide nanoparticles alleviate arsenic induced stunting in Vigna radiata[J].Ecotoxicology and Environmental Safety, 2019, 183(Nov.).

[2 Kabir A H , Akther M S , Skalicky M ,et al.Downregulation of Zn-transporters along with Fe and redox imbalance causes growth and photosynthetic disturbance in Zn-deficient tomato[J].Scientific Reports, 2021, 11(1).

[3] Ojeda M , Schaffer B , Davies F S .Root and Leaf Ferric Chelate Reductase Activity in Pond Apple and Soursop[J].Journal of Plant Nutrition, 2005, 27(8):1381-1393.

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