Pyrroline-5-carboxylic acid reductase (P5CR) activity Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: NA0144

Size:100T/48S

Components:

Extract solution I:60 mL×1. Storage at 2-8℃.

Extract solution II:600 μL×1. Storage at -20℃. Reagents are volatile, seal as soon as possible after use and put back to -20℃ for storage. The preparation of the extraction solution: according to the sample volume the extraction solution I extraction solution II = 0.01mL: 0.99mL (1T) ratio of the preparation of the extraction solution, ready to use, forbidden to prepare in advance.

Reagent I: 5 mL×1. Storage at 2-8℃.

Reagent II: 10mL×1. Storage at 2-8℃.

Reagent III: powder×1. Storage at -20℃. Add 5.6mL of reagent I before use and mix thoroughly. The unused reagents can be stored in separate packages at -20℃ for 4 weeks, avoid repeated freezing and thawing.

Reagent IV:4mL×1. Storage at 2-8℃.

Standard: powder×1, storage at -20℃. Add 1.4 mL distilled water before use0. 2 μmol/mL NADH standard solution. The unused reagents should be stored in separate containers at -20℃ for 2 weeks, avoiding repeated freezing and thawing during the period. Before use, take 100 μL of 2 μmol/mL NADH standard solution in EP tube, add 700 μL of distilled water to dissolve fully, and prepare 0.25 μmol/mL NADH standard solution for use.

Product Description:

Pyrroline-5-carboxylate reductase (P5CR) is an important housekeeping protein widely found in prokaryotes and eukaryotes. In the presence of NAD(P)H, pyrroline-5-carboxylic acid (P5C) is converted to proline by pyrroline-5-carboxylate reductase, and P5CR has also been found to be involved in the metabolism of thioproline in E. coli.

Thioproline is dehydrogenated by pyrroline-5-carboxylate reductase and is accompanied by the conversion of NAD to NADH. In the presence of 1-mPMS, WST-1 reacts with NADH to produce water-soluble formazan with a characteristic absorption peak at 450 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, water bath/constant incubator, adjustable pipette, analytical balance, mortar/homogenizer/cell sonicator , micro glass cuvettes/96 well plates, distilled water and ice.

Procedure

I. Extraction of crude enzyme solution:

a. Tissue

The ratio of tissue mass (g): the volume of Extract solution (mL) is 1: 5~10 (it is suggested to take about 0.1 g of tissue and add 1mL of Extract solution), ice-bath homogenate. Centrifuge at 8000 g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.

b. Bacteria or cells

The ratio of bacteria/cell amount (10^4): the volume of Extract solution (mL) is 500~1000:1(it is suggested to take about 5 million bacteria/cells and add 1 mL of Extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200 W, work time 3 s，interval 10 s，repeat 30 times). Centrifuge at 8000g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.

c. Serum (plasma) sample: Detect sample directly. (If the solution is turbid, centrifuge to take the supernatant and then measure)

II. Determination procedure

a. Preheat the spectrophotometer 30 minutes, adjust wavelength to 450 nm, set zero with distilled water.

b. Then operate according to the following table.

Reagent name （μL）	Test tube(T)	Control tube(C)	Standard tube(S)	Blank tube(B)
Reagent II	90	90	90	90
Reagent III	90	-	-	-
distilled water	-	90	90	110
Reagent IV	20	20	20	20
Standard solution	-	-	20	
sample	20	20	-	-
Mix thoroughly, react at 37℃ for 30min, take 200μL in a micro glass cuvettes/96 well plates, and measure the absorbance at 450nm. It was recorded as At, Ac, As and Ab. ΔAt = At - Ac, ΔAs= As – Ab.(Standard and blank tubes should be done only 1-2 times.)				

III. Calculation formula

(1) Calculation by sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 nmol NADH per milligram protein of tissue per minute.

P5CR activity (U/mg prot) = (ΔAt × Cs ÷ ΔAs) × Vs ÷ (Cpr × Vs) ÷ T × 10^3 × F

= 8.333 × Δt ÷ Δ s÷ Cpr × F

(2) Calculation by sample mass

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 nmol NADH per gram of tissue per minute.

P5CR activity (U/g mass) = (ΔAt × Cs÷ ΔAs) × Vs ÷ (W ÷ Vst× Vs) ÷ T × 10^3 × F

= 8.333 × Δt ÷Δs ÷ W × F

(3) Calculation by number of bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 nmol NADH per 10^4 cells of bacteria or cells per minute.

P5CR activity (U/10^4 cell) = (ΔAt × Cs ÷ ΔAs) × Vs ÷ (500 ÷ Vst × Vs) ÷ T × 10^3 × F

= 0.0167 × Δt ÷ Δs × F

(4) Calculated by volume of serum (plasma) and other liquids

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 nmol NADH per milliliter of serum (plasma) per minute.

P5CR activity (U/mL) = (ΔAt× Cs ÷ ΔAs) × Vs ÷ Vs÷ T × 10^3 × F = 8.333× Δt ÷ Δ s × F

C standard: concentration of NADH standard solution, 0.25 μmol/mL; Vs: volume of sample added to the reaction system, 0.02 mL; Vst: volume of extract added, 1 mL; T: reaction time, 30 min; Cpr: protein concentration, mg/mL; W: sample mass, g.

Note:

1. If At is greater than 1.8 or ΔAt is greater than 1, reduce the sample volume or shorten the reaction time; if ΔAt is less than 0.01, increase the sample volume or extend the reaction time to 1h or longer. Note the simultaneous modification of the calculation formula.

Experimental example:

1. Take 0.0993 g of orange leaves, added to the extract for ice bath homogenization, operated according to the assay procedure, and measured to calculate ΔAt = At - Ac = =0.318-0.288=0.03, ΔAs= As - Ab=0.538-0.181=0.357, brought into the equation to calculate.
   P5CR activity (U/g mass) = 8.333×0.03÷0.357÷0.0993=7.052 U U/g
2. Take 0.1006 g of mouse kidney tissue, added to the extract for ice bath homogenization, operated according to the assay procedure, and measured to calculate ΔAt = At - Ac= 0.369-0.274=0.095, ΔAs = As - Ab = 0.538-0.181=0.357, brought into the equation to calculate.
   P5CR activity (U/g mass) = 8.333×0.095÷0.357÷1.006=22.042 U/g
3. Take 0.02 mL of sheep serum and operated according to the assay procedure and measured to calculate ΔAt = At - Ac = 0.299-0.146=0.153, ΔAs = As- Ab = 0.538-0.181=0.357, brought into the formula to calculate.
   P5CR activity (U/mL) =8.333×0.153÷0.357=3.571 U/mL

Related products:

NA0845/NA0603 Proline (PRO) Content Assay Kit

NA0288/NA0287 1-Pyrroline-5-carboxylic acid synthase (P5CS) activity Assay Kit