Sorbitol Dehydrogenase (SDH) Activity Assay Kit

Note: The reagents of this product are subject to change. Please note and strictly follow this instruction.

Operation Equipment: Spectrophotometer

Cat No: BC2530

Size:50T/48S

Components:

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

Reagent I: Powder×5. Storage at 2-8℃. Take 10 mL of Reagent V and add it to 1 bottle of Reagent I powder, dissolve and add a reagent IV, Prepare the reagent before use, and it will deteriorate in 24 hours.

Reagent II: 12mL×1. Storage at 2-8℃.

Reagent III: 12mL×1. Storage at 2-8℃.

Reagent IV: Powder×5. Storage at -20℃.

Reagent V: 55mL×1. Storage at 2-8℃.

Standard: Powder×1. Storage at -20℃. Add 1.4 mL of distilled water before use, which is 10 μmol/mL NADH standard. Reagents can be stored at -20℃ for 4 weeks, avoiding repeated freezing and thawing.

Product Description:

SDH (EC 1.1.1.14) catalyzes the dehydrogenation of sorbitol to fructose, which is one of the key enzymes to regulate the content of sorbitol in vivo.

SDH catalyzes the dehydrogenation of sorbitol to fructose, and the reduction of NAD^+ to NADH. The generated NADH can transfer electrons to NBT to generate purple hairpin. According to this principle, SDH activity can be calculated.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, desk centrifuge, water bath/ constant temperature incubator, transferpettor, sonicator, 1 mL glass cuvette, mortar/homogenizer, ice, distilled water.

Procedure

I. Sample preparation:

a. Bacteria or cells

Collecting bacteria/cells into the centrifuge tube. The supernatant is discarded after centrifugation. 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/cell and add 1 mL of Extract solution). Bacteria/cell is splitted by ultrasonication(placed on ice, power 200W, work time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000×g for 10 minutes at 4℃, and the supernatant is used for test.

b. Tissue

The ratio of tissue mass (g): the volume of Extract solution (mL) = 1: 5~10 (it is suggested to take about 0.1 g of tissue and add 1 mL of Extract solution), ice-bath homogenate. Centrifuge at

8000 ×g for 10 minutes at 4℃, and the supernatant is used for test.

c. Serum (plasma) sample: Detect sample directly.

II. Determination procedure

a. Preheat the spectrophotometer for 30 minutes, adjust wavelength to 570 nm, set zero with distilled water.

b. standard solution

The 10 μmol/mL NADP is respectively diluted 1, 0.9, 0.8, 0.7, 0.6,0.5, 0.4 and 0.3 μmol/mL NADP standard solution by distilled water. Then operate according to the following table.

Reagent name (μL)	Standard tube (S)	Blank tube (B)
Standard solution	100	-
Distilled water	-	100
Reagent II	150	150
Reagent III	150	150
Reagent IV	600	600
After mixing, place it at room temperature for 20 minutes, measure the absorbance of standard tube and blank tube at 570 nm respectively, record it as A_S, A_B, calculate $\Delta A_S=A_S-A_B$. The standard curve only needs to be done 1-2 times.		

c. Sample Test

Reagent name (μL)	Test tube (T)
Sample	100
Reagent II	150
Reagent III	150
Reagent IV	600

Add the above reagents to the 1 mL glass cuvette in sequence, start timing at the same time of adding samples, record the initial absorbance A1 at 10 s. Put the cuvette together with the reaction solution into a water bath at 37℃(mammal) or 25℃ (other species) for 3 minutes after color comparison, take out the cuvette quickly and dry it. Determinate at 570 nm, record the absorbance at 3min10s A2, calculate ΔA_T= A1-A2. (The whole experiment should be protected from light)

III. SDH Calculations

1. Drawing of standard curve

According to the concentration of the standard tube (x, μmol/mL) and the absorbance ΔA standard (y, ΔA standard), establish a standard curve. According to the standard curve, bring the ΔA assay (y, ΔA assay) into the formula to calculate the sample concentration (x, μmol/mL)

2. Calculate the activity of SDH

(1) Serum (plasma) sample SDH activity

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of NADH in the reaction system per minute every milliliter serum (plasma) .

SDH (U/mL)=1000×x×V_S÷V_S÷T=333×x

(2) Tissue, bacteria or cultured cells SDH activity

a. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of NADH in the reaction system per minute every milligram protein.

SDH (U/mg prot)= 1000×x×V_S÷(V_S×Cpr)÷T=333×x÷Cpr

b. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of NADH in the reaction system per minute every gram sample.

SDH (U/g fresh wight) =1000×x×V_S÷(W×V_S÷V_{STV}) ÷T=333×x÷W。

c. Cell amount

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of NADH in the reaction system per minute every 10^4 cells.

SDH (U/10^4 cell)=1000×x×V_S÷(500×V_S÷V_{STV}) ÷T=0.666×x

V_S: Add the volume of sample, 0.1 mL;

V_{STV}: The volume of extract, 1 mL;

T: Reaction time, 3 minutes;

Cpr: The concentration of sample protein, mg/mL;

W: Sample weight, g;

500: Total number of bacteria or cells, 5 million;

10^3: 1 μmol=10^3 nmol.

Note:

1. When the absorbance value of spectrophotometer is greater than 0.7, it is recommended to measure after dilution.
2. Place the sample and working solution on the ice during the determination to avoid denaturation and deactivation.
3. The temperature of the reaction solution in the cuvette must be kept at 37℃ or 25℃. Take a small beaker and put it into a certain amount of 37℃ or 25℃ distilled water. Put the beaker into a 37℃ or 25℃ water bath. Put the cuvette and reaction solution into the beaker during the reaction.

References:

Aguayo M F, Ampuero D, Mandujano P, et al. Sorbitol dehydrogenase is a cytosolic protein required for sorbitol metabolism in Arabidopsis thaliana[J]. Plant science, 2013, 205: 63-75.

Related Products:

BC2450/BC2455 Plant Tissue Fructose Content Assay Kit

BC2540/BC2545 Cellulase(CL) Activity Assay Kit

BC2510/BC2515 Trehalase Activity Assay Kit

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