Sorbitol Dehydrogenase(SDH) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC2535

Size:100T/96S

Components:

Extract solution: 110 mL×1. Storage at 2-8℃.

Reagent I: Powder×5. Storage at 2-8℃. Take 3.4 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: 4mL×1. Storage at 2-8℃.

Reagent III: 4mL×1. Storage at 2-8℃.

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

Reagent V: 25mL×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/Microplate reader, desk centrifuge, water bath/ constant temperature incubator, transferpettor, micro glass cuvette/96 well flat-bottom plate, 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 split 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) is 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 min at 4℃, and the supernatant is used for test.

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

II. Determination procedure

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

b. Standard solution

The 10 μmol/mL NADP is respectively diluted 1.5, 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	20	-
Distilled water	-	20
Reagent II	30	30
Reagent III	30	30
Reagent IV	120	120
After mixing, place it at room temperature for 20 minutes, take 200 μL in micro glass cuvette/96 well plate and 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	20
Reagent II	30
Reagent III	30
Reagent IV	120

Add the above reagents to the micro glass cuvette/96 well plate in sequence, start timing at the same time of adding samples, record the initial absorbance A1 at 10s. 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.

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 sample volume, 0.02 mL;

V_{STV}: Extract volume, 1 mL;

T: Reaction time, 3 minutes;

Cpr: Sample protein concentration, 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 is greater than 0.7, it is recommended to dilute the sample with the extract solution and measure it, multiply the dilution factor in the calculation formula.
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
BC2520/BC2525	Sorbitol Content Detection Kit