Sorbitol Dehydrogenase(SDH) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: NA0448

Size:100T/96S

Components:

Extract solution: 65 mL×2. Storage at 4℃.

Reagent I: Powder×1. Storage at 4℃. Add 17 mL of Reagent V into Reagent I powder, dissolve and store at 4℃.

Reagent II: Powder×1. Storage at -20℃. Before use, add 4 mL of Reagent V, after fully dissolved, 0.8 mL of per bottle can be sub packed and stored at -20℃;

Reagent III: Powder×1. Storage at -20℃. Before use, add 4 mL of Reagent V, after fully dissolved, 0.8 mL of per bottle can be sub packed and stored at -20℃;

Reagent IV: Powder×5. Storage at -20℃. Add 3.4 mL of Reagent I before use, which is the working solution. Prepared when the solution will be used, and it is deteriorated in 24 hours.

Reagent V: 30 mL×1. Storage at 4℃.

Standard: Powder×1. Storage at -20℃. Add 1.4 mL of distilled water before use, which is 10 μmol/mL NADH standard. It can be stored for one week at -20℃.

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, 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, 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, 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 $\Delta A_S=A_S-A_B$.		

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

Take ΔA_S as y-axis, take standard solution concentration as x-axis, draw standard curve, get standard equation y=kx+b, bring ΔA_T into equation to get 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 mL 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 mg 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 g 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 of spectrophotometer is greater than 0.7 or that of microplate reader is greater than 0.5, 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：

NA0695/NA0454 Plant Tissue Fructose Content Assay Kit

NA0688/NA0447 Cellulase(CL) Activity Assay Kit

NA0691/NA0450 Trehalase Activity Assay Kit

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