(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Sorbitol dehydrogenase (SDH) Activity Assay Kit

Catalog No.: BC00137

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Sorbitol dehydrogenase (SDH) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Bacteria, cells, tissues, serum, plasma
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (340 nm)

Product Introduction

SDH catalyzes the dehydrogenation of sorbitol to produce fructose and is one of the key enzymes regulating sorbitol levels in organisms.

Principle

SDH catalyzes the dehydrogenation of sorbitol to produce fructose, while simultaneously reducing NAD+ to NADH. The activity of SDH can be calculated by measuring the rate of increase in absorbance at 340 nm.

Components

Components	Size	Storage
Extract	Liquid 60 mL × 1 bottle	Store at 4℃
Reagent 1	Liquid 20 mL × 1 bottle	Store at 4℃
Reagent 2	Powder × 1 bottle	Store at 4℃
Reagent 3	Powder × 1 bottle	Store at -20℃

Note: Add 15mL of distilled water to each bottle of reagent 2 before use. Store any unused reagent at 4℃.

Add 15mL of distilled water to each bottle of reagent 3 before use. Store any unused reagent at -20℃.

Storage

This kit is stored under the specified conditions upon receipt and has a shelf life of 3 months.

Preparation before the experiment

Sample processing

1. Bacteria or cultured cells: First, collect bacteria or cells into centrifuge tubes, centrifuge, and discard the supernatant; according to the ratio of bacteria or cell number (10^4) : extraction liquid volume (mL) of 500-1000:1 (it is recommended to add 1 mL of extraction liquid for 5 million bacteria or cells), sonicate the bacteria or cells (ice bath, power 20% or 200W, sonicate for 3 seconds, with 10-second intervals, repeat 30 times); centrifuge at 8000g, 4°C for 10 minutes, collect the supernatant, and place it on ice for testing.
2. Tissue: Homogenize the tissue at a ratio of 1:5-10 (tissue mass (g): extraction liquid volume (mL) – it is recommended to weigh approximately 0.1g of tissue and add 1mL of extraction liquid) in an ice bath. Centrifuge at 8000g, 4℃ for 10min, and collect the supernatant on ice for analysis.
3. Serum (plasma) samples: direct detection.

Operation process

Reagents	Test
Reagent 1 (μL)	400
Reagent 2 (μL)	300
Reagent 3 (μL)	300
Mix well and incubate in a water bath at 37°C (mammals) or 25°C (other species) for 5 minutes.	
Sample(μL)	50

Add the above reagents to a 1mL quartz cuvette in sequence. While adding the sample, start timing and record the initial absorbance A1 at 20 seconds and the absorbance A2 at 2 minutes and 20 seconds.

Result calculation

1. Calculation of SDH activity in serum (plasma):

Unit definition: One unit of enzyme activity is defined as 1 nmol of NADH generated per minute per milliliter of serum (plasma).

$$\text{SDH Activity（U/mL）} = \frac{\Delta A \times V_{reaction\_total} \times 10^9}{\varepsilon \times d} \div V_{sample} \div T = 1688 \times \Delta A$$

2、Calculation of SDH activity in tissues, bacteria, or cells:

(1) Calculated based on protein concentration:

Unit definition: One unit of enzyme activity is defined as 1 nmol of NADH produced per milligram of protein per minute.

$$\text{SDH Activity（U/mgprot）} = \frac{\Delta A \times V_{reaction\_total} \times 10^9}{\varepsilon \times d} \div (V_{sample} \times Cpr) \div T = 1688 \times \Delta A \div Cpr$$

(2) Calculated based on sample fresh weight:

Unit definition: One unit of enzyme activity is defined as 1 nmol of NADH produced per minute per gram of tissue.

$$\text{SDH Activity（U/g）} = \frac{\Delta A \times V_{reaction\_total} \times 10^9}{\varepsilon \times d} \div (V_{sample} \times \frac{W}{V_{sample\_total}}) \div T = 1688 \times \Delta A \div Cpr$$

(3) Calculated by bacterial or cell density:

Unit definition: One unit of enzyme activity is defined as 1 nmol of NADH produced per minute by 10,000 bacteria or cells.

$$\text{SDH Activity（U/10}^4\text{cells）} = \frac{\Delta A \times V_{reaction\_total} \times 10^9}{\varepsilon \times d} \div (V_{sample} \times \frac{\text{Cell count}}{V_{sample\_total}}) \div T = 1688 \times \Delta A \div Cpr$$

Annotation

$V_{reaction\_total}$: total volume of the reaction system, 1.05×10^{-3}L;

ε: NADH molar extinction coefficient, 6.22×10^3 L /mol/cm;

d: Cuvette optical path length, 1 cm;

V sample: Add 0.05 mL of sample volume;

V_{sample_total}: Volume of extract added, mL;

T: Reaction time, 2 min;

Cpr: Sample protein concentration, mg/mL;

W: Sample mass, g;

Cell count: Total number of bacteria or cells, 10^4

Precautions

This product is for research use only and must not be used for clinical diagnosis. Do not take it.