ELK Biotechnology

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Sorbitol dehydrogenase Assay Kit Instruction

（BC051 50T/48S）

一、Assay significance

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

二、Assay principle

SDH catalyzes the dehydrogenation of Sorbitol to fructose and the reduction of NAD + to Nadh. SDH activity can be calculated by measuring the increasing rate of absorbance at 340nm.

三、Reagents and tools required but not supplied

① Visible spectrophotometer, Activity (Wavelength: 545nm)

② High speed refrigerated centrifuge and tubes, Water bath tank

③ Adjustable pipette (5-1000μl) and Tips

④ 1mL quartz cuvettes, Mortar

⑤ Ice and Distilled water

四、Reagents composition:（50T/48S）

Extract Solution: Liquid 60ml×1 bottle,store at 4°C;

Reagent 1: Liquid 20ml×1 bottle,store at 4°C;

Reagent 2: Powder×1 bottle,store at 4°C;Before use, add 15ml distilled water and dissolve the mixture thoroughly,store the remaining reagents at 4°C.

Reagent 3: Powder×1 bottle,store at -20°C;Before use, add 15ml distilled water and dissolve the mixture thoroughly,store the remaining reagents at -20°C.

五、Operation Procedure:

1、Extraction of crude enzyme:

（1）**Bacteria、Cells:** Collect the bacteria or cells into the centrifuge tube ,and then discard the supernatant after centrifugation. According to the number of bacteria or cells (10^4) : Extract Solution volume (ml)500 ~ 1000:1 ratio (5 million bacteria or cells are

recommended to add 1ml extract solution), the ice bath ultrasonic wave was broken (20% power or 200W, ultrasonic 3s, interval 10s, repeat 30 times) , and 8000g centrifuged at 4°C for 10min, take the supernatant and placed on the ice to be tested.

（2）**Tissue**：According to the Tissue mass (g) : the volume ratio of extract solution 1:5 ~ 10(about 0.1g tissue is recommended, and add 1mLextract solution) , ice bath homogenization is carried out. 8000g centrifuged at 4°C for 10min,take the supernatant and placed on ice for test.

（3）**Serum (Plasma) and other fluids sample:** Direct test.

2、Operation table：

Reagent	Assay
Reagent 1（μL）	400
Reagent 2（μL）	300
Reagent 3（μL）	300
Mix well and incubate at 37 °C (Mammal) or 25 °C (other species) for 5 minutes	
Sample（μL）	50
Add the above reagents to 1ml quartz colorimetric dish in turn;Timing when you add the sample,recorde the initial absorbance A_1 at 20s and A_2 at 140s,calculate $\triangle A=A_2-A_1$.	

六、Calculate：

1、SDH in serum（Plasma）

Unit definition：Production of 1nmol NADH per milliliter of serum (Plasma) per minute was defined as an enzyme activity unit.

$$SDH\ \underset{(\mathrm{U/ml})}{\text{Activity}} = \frac{\triangle A \times V_{\text{Total Volume}} \times 10^9}{(\varepsilon \times \mathrm{d})} \div V_{\text{Sample}} \div T = 1688 \times \triangle A$$

2、SDH in tissue、bacteria or cells

⑴、Calculated by sample protein concentration

Unit definition：Production of 1nmol NADH per mg protein per minute was defined as an enzyme activity unit.

$$SDH\ \underset{(\mathrm{U/mgprot})}{\text{Activity}} = \frac{\triangle A \times V_{\text{Total Volume}} \times 10^9}{(\varepsilon \times \mathrm{d})} \div (V_{\text{Sample}} \times \mathrm{Cpr}) \div T = 1688 \times \triangle A \div \mathrm{Cpr}$$

⑵、Calculated by sample fresh weight

Unit definition：Production of 1nmol NADH per gram tissue per minute was defined as an enzyme activity unit.

$$SDH\ \text{Activity}\ (\text{U/g}\ \text{Tissue}) = \frac{\triangle A \times V_{\text{Total Volume}} \times 10^9}{(\varepsilon \times \text{d})} \div \left(V_{\text{Sample}} \times \frac{\text{W}}{V_{\text{Total Sample}}}\right) \div T = 1688 \times \triangle A \div \frac{\text{W}}{V_{\text{Total Sample}}}$$

⑶、Calculated by Cells or Bacteria density

Unit definition：Production of 1nmol NADH per 104 bacteria or cells per minute was defined as an enzyme activity unit.

$\mathbf{V_{sample}}$:Volume of supernatant added to reaction system（mL）,0.05mL

$\mathbf{V_{Total\ samples}}$:The volume of the supernatant, 1mL;

$\mathbf{V}_{\text{Total volume}}$:Total volume of reaction system（L）,1.05mL；

T：Reaction time（min）,2min

ε :Molar absorptivity of NADH,6.22×10^3 L / mol /cm;

d :light path（cm）,1cm

W: Sample quality,（g）

Cpr : Sample protein concentration,mg/mL

500：Bacteria or cell numbers,5 million