Sorbitol Dehydrogenase Microplate Assay Kit

Basic information:

Catalog No.: UAK1101 **Size:** 100 Assays

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Sorbitol dehydrogenase (SDH), found in organisms from bacteria to humans, converts sorbitol, the sugar alcohol form of glucose, to fructose, and the zinc-dependent reaction uses NAD^+ as a cofactor. Organs abundant in SDH activity include the liver, kidney, lens and seminal vesicle. SDH and aldose reductase (AR) form the polyol pathway that interconverts glucose and fructose. Since SDH activity promotes the formation of NADH, the redox change induced by elevated SDH may have a pathogenic role in certain disease state, making SDH a potential therapeutic target. In addition, SDH has a close evolutionary relationship with alcohol dehydrogenase (ADH).

The assay is initiated with the enzymatic hydrolysis of the sorbitol by sorbitol dehydrogenase. The enzyme catalysed reaction products NADH, can be measured at a colorimetric readout at 340 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	-20 °C
Substrate Diluent	20 ml x 1	4 °C
Standard	Powder x 1	-20 °C
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Note:

***Substrate**: add 19 ml Substrate Diluent to dissolve before use.*

***Standard**: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.*

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 10,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum, plasma and other biological fluids samples

Detect directly.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 μl	--
Distilled water	--	--	200 μl
Sample	10 μl	--	--
Substrate	190 μl	--	--
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.			

***Note**: if the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time.*

VI. CALCULATION

Unit Definition: One unit of sorbitol dehydrogenase activity is defined as the enzyme that generates 1 nmol of NADH per minute.

1. According to the protein concentration of sample

$$SDH\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 4000 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$SDH\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$SDH\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

$$SDH\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 4000 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 400 nmol/ml;

$V_{Standard}$: the volume of standard, 200 µl = 0.2 ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

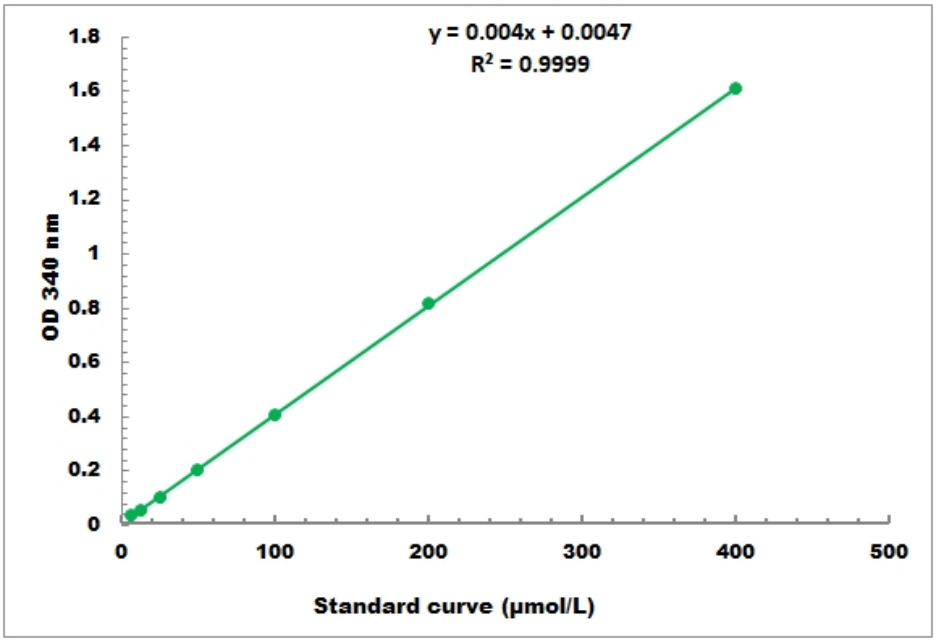
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4 µmol/L - 400 µmol/L