Sorbitol Dehydrogenase

Microplate Assay Kit

User Manual

Catalog # CAK1101

(Version 1.4D)

Detection and Quantification of Sorbitol Dehydrogenase (SDH) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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 450 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	10 ml x 1	4 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	-20 °C
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Note:

Substrate: add 9 ml Substrate Diluent to dissolve before use.

Dye Reagent A: add 9 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.3 ml into 0.7 ml distilled water, the concentration will be 600 µmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel 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%, sonicate 3s, interval 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	Control	Standard	Blank
Standard	--		100 µl	--
Distilled water	--		--	100 µl
Substrate	90 µl	90 µl	--	--
Sample	10 µl		--	--
Dye Reagent A	90 µl	90 µl	90 µl	90 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl
Mix, incubate at room temperature for 5 minutes, record absorbance measured at 450 nm.				

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

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

1. According to the protein concentration of sample

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

$$= 1.2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 1.2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

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

$$= 1.2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

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

$$= 1.2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 600 μmol/L = 0.6 μmol/ml;

$V_{Standard}$: the volume of standard, 100 μl = 0.1 ml;

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

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

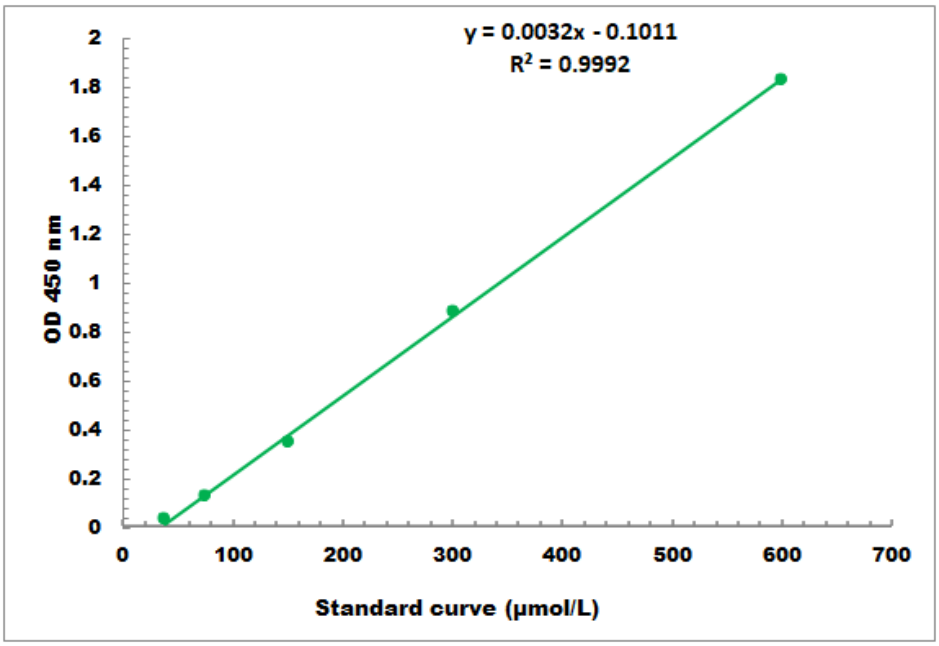
V_{Sample}: the volume of sample, 0.01 ml;

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

T: the reaction time, 5 minutes.

VII. TYPICAL DATA

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



Detection Range: 30 μmol/L - 600 μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES