Sorbitol Dehydrogenase Activity Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1101

(Version 1.5E)

Detection and Quantification ofSorbitol Dehydrogenase(SDH) ActivityinUrine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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 ofglucose, 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).

Sorbitol Dehydrogenase Activity Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring sorbitol dehydrogenase activity levels in a variety of samples.The assay is initiated with the enzymatic hydrolysis of the sorbitol bysorbitol dehydrogenase. The enzyme catalyzed reaction productsNADH, can be measured at a colorimetric readout at 450 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	11 ml x 1	4 °C
Coenzyme Diluent	1 ml x 1	4 °C
Coenzyme	Powder x 1	4 °C, keep in dark
Substrate	Powder x 1	4 °C
Dye ReagentA	Powder x 1	4 °C, keep in dark
Dye Reagent B	1 ml x 1	4 °C, keep in dark
Standard	Powder x 1	-20 °C
Positive Control	Powder x 1	-20 °C
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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
9. Convection oven

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 2 mmol/L of standardstock solution, store at 4°C for 1 day or -20°C for 3daysafter reconstitution.Then dilute to400 µmol/Lstandard top solutionby adding0.2 ml stock solution into 0.8 mldistilled water**.**Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be400/200/100/50/25/12.5/6.25µmol/L.

Coenzyme: Briefly centrifuge prior to opening.Add 1 mlCoenzyme Diluent to dissolve before use.Aliquot & store at -80 °C.Use within 1 month.

Substrate: Briefly centrifuge prior to opening. Add 1 ml Reaction Bufferto dissolve before use. Store at 4 °C for 2 weeks or -20°C for 6 monthsafter reconstitution.

Dye Reagent A: Add 8 mldistilled water to dissolve before use.Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Assay BuffertogeneratePositive Control (4X).Then dilute by adding 0.1 ml Positive Control(4X) into 0.3 mlAssay Buffer, mix.Aliquot & store at -80 °C.Use within 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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

Weighout 0.1 g tissue, homogenize with 1 mlAssay 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.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents intothe microplate:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Reaction Buffer	80 µl	80 µl	80 µl	--	--
Coenzyme	10 µl	10 µl	10 µl	--	--
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	--	10 µl	110 µl
Positive Control	--	--	10 µl	--	--
Substrate	10 µl	10 µl	10 µl	--	--
Standard	--	--	--	100 µl	--
Dye Reagent A	80 µl	80 µl	80 µl	80 µl	80 µl
Dye ReagentB	10 µl	10 µl	10 µl	10 µl	10 µl
Mix, put it into the convection oven,37°C for 30 minutes, record absorbance measured at 450 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VI. CALCULATION

Unit Definition: One unit ofsorbitol dehydrogenaseactivity is defined as the enzyme thatgenerates 1μmol of NADH per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

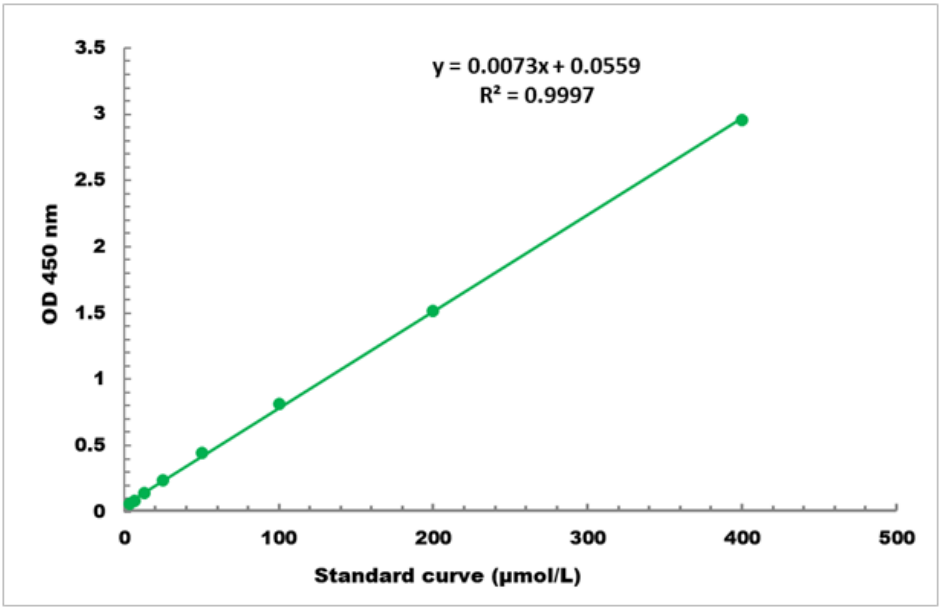
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

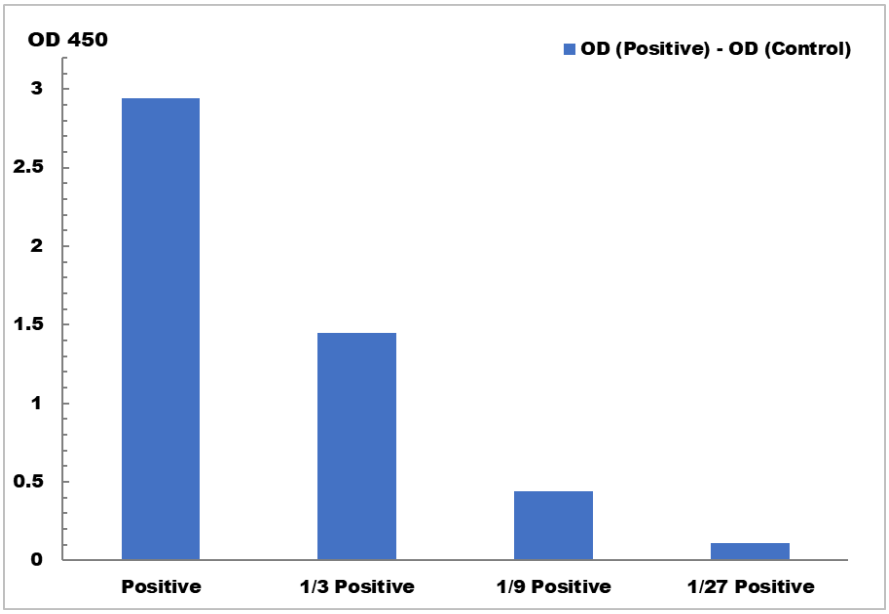
T: the reaction time, minute

VII. TYPICAL DATA

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



Detection Range: 4μmol/L - 400 μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration