Aldehyde DehydrogenaseActivity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1040

(Version 1.4E)

Detection and Quantification of Aldehyde Dehydrogenase (ALDH) 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.

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I. INTRODUCTION

In mammals, ethanol is metabolized mainly in the liver by alcohol dehydrogenase (ADH), which oxidizes ethanol to acetaldehyde. Acetaldehyde, a toxic metabolite responsible for the miserable effects of hangovers, is further oxidized to acetate by aldehyde dehydrogenase (ALDH). ALDH belongs to a large family of aldehyde dehydrogenases that can be found in many tissues of the body, but are at the highest concentrations in the liver.

Aldehyde DehydrogenaseActivity ColorimetricMicroplate Assay Kit provides a simple and direct procedure for measuringAldehyde Dehydrogenase activity in a variety of samples.In this colorimetricALDH quantification assay, ALDH reduces NAD to NADH, which then interacts with a specific probe to produce a color. The rate of increase in the absorbency at 450 nm, is a measure of ALDH activity.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	16 mlx 1	4 °C
Coenzyme	Powderx 1	-20 °C, keep in dark
Substrate	0.5 ml x 1	4 °C, keep in dark
Substrate Diluent	9 ml x 1	4 °C
Dye ReagentA	Powder x 1	4 °C, keep in dark
Dye Reagent B	2 mlx 1	-20 °C, keep in dark
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

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. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening. Dissolvein1 ml distilled waterto generatestandard stock solution.Dilute to 600 µmol/L top standardsolutionby adding 0.3 ml stock solution into 0.7 mldistilled water.Then perform 2-fold serial dilutions of the top standardsolution by distilled waterto make the standard curve. The concentration of standard curve could be 600/300/150/75/37.5 µmol/L.Store at -20 °C for 1 month or 4°C for 3 days.

Dye Reagent A: Add 10 mldistilled water to dissolve before use, mix, store at 4°C for 7 days or -20°Cfor 1 month.

Coenzyme: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use, store at 4°C for 7 days or -20°Cfor 1 month.

Substrate Working Solution:Prepareready-to-use SubstrateWorking Solution by mixing Substrate and Substrate Diluent ata ratio of 11:189 (v/v). Keep in dark and use immediately.

Positive Control: Briefly centrifuge prior to opening. Dissolvein0.45 ml Assay Bufferto generate stock solution. Dilute the stock solution 10-fold using Assay Buffer to prepare the Positive Control working solution (eg. 10 µlto 90 µlAssay Buffer).Store at -80 °C for 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 8000g 4°C for 10minutes, 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 8000g 4°C for 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquid samples

Add50 µlsample into 100 µlAssay Buffer, mix and incubate at room temperature for 10 minutes. Keep it on ice for detection.

VI. ASSAY PROCEDURE

Add samples to a paired set of wells in a 96-well plate as Sample and Control.

Add following reagents in the microplate:

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Reaction Buffer	70 µl	90 µl	--	--	70 µl
Coenzyme	10 µl	--	--	--	10 µl
Substrate Working Solution	10 µl	--	--	--	10 µl
Sample	50 µl	50 µl	--	--	--
Positive Control	--	--	--	--	50 µl
Standard	--	--	100 µl	--	--
Distilled water	--	--	40 µl	140 µl	--
Mix, incubate at 37°C for 15 minutes					
Dye Reagent A	50 µl	50 µl	50 µl	50 µl	50 µl
Dye ReagentB	10 µl	10 µl	10 µl	10 µl	10 µl
Mix, incubate at room temperature for 15 minutes recordabsorbance 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.

VII. CALCULATION

Unit Definition: One unit of ALDH activity is defined asthe enzyme products 1 μmol of NADH per minute.

1. According to the slope of the standard curve

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

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

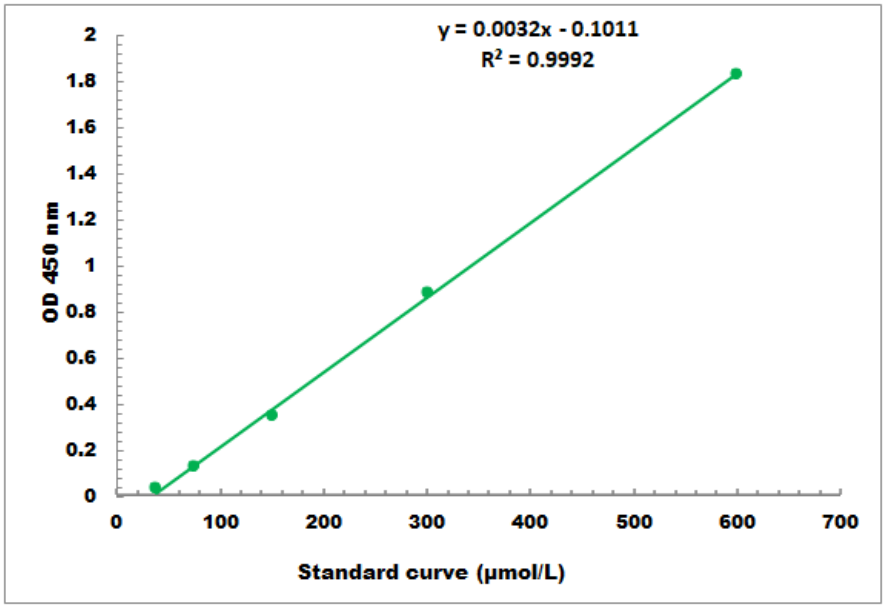
V_{Sample}: the volume of sample in assay procedure, mL

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

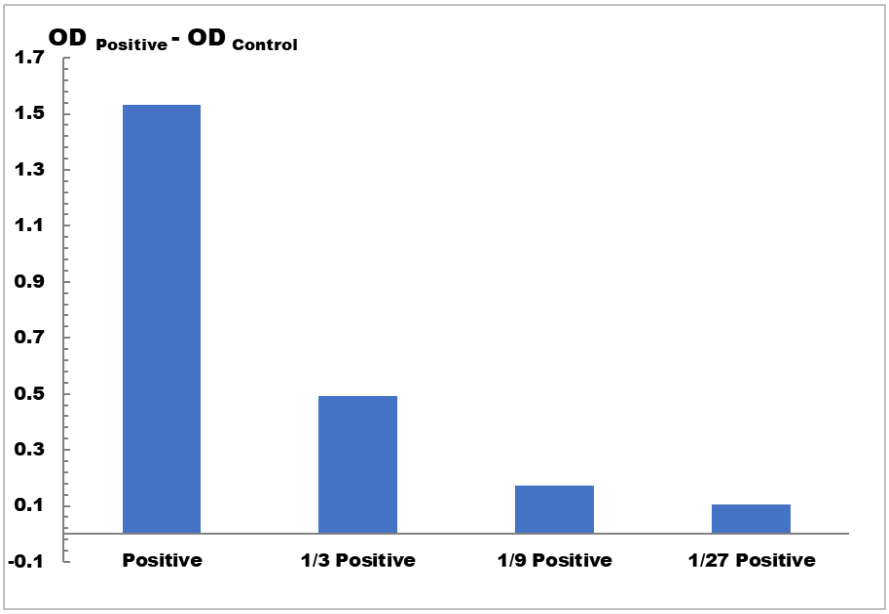
T: the reaction time, minute

VIII. TYPICAL DATA

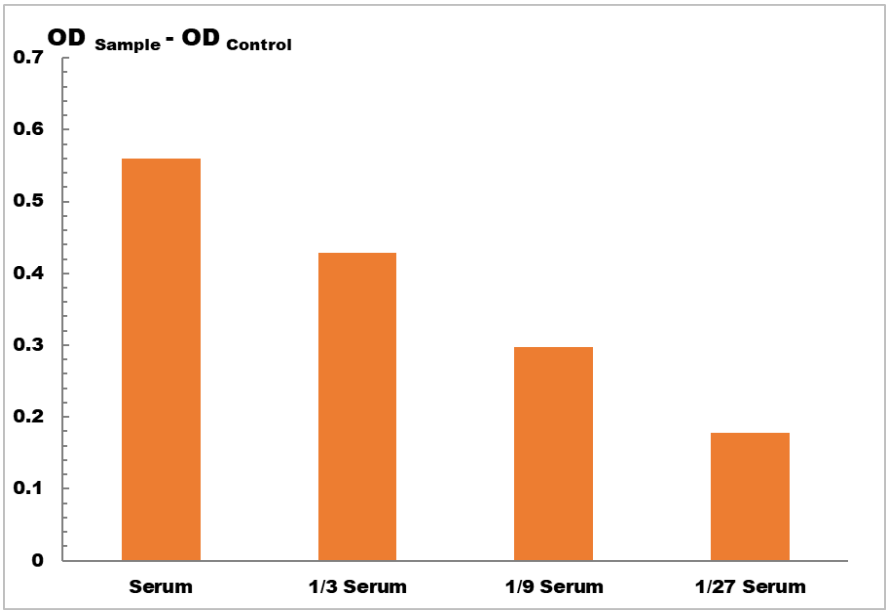
The standard curve is for demonstration only. A standard curve must be run with each assay.



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



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



Determination of aldehyde dehydrogenaseactivity in serum