Alcohol Dehydrogenase Microplate Assay Kit

Catalog # AS0084

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

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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I. INTRODUCTION

Alcohol dehydrogenases (ADH) are a family of enzymes that catalyzes the conversion of alcohols to aldehydes, with the concomitant reduction of NAD+ to NADH. In humans, there are nine isozymes of ADH, with the majority of ADH activity occurring in the liver. ADH family members are the primary enzymes involved in alcohol detoxification. Genetic variations in ADH enzymes result in differences in ADH activity and tolerances for alcohol, and may regulate susceptibility to alcoholism.

The reaction velocity is determined by the rate of absorbance at 340 nm resulting from reduction of NADH is measured.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Substrate	4 mlx 1	4 °C
Coenzyme	Powderx 1	-20 °C
Coenzyme Diluent	15 ml x 1	4 °C
Standard	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

Enzyme: add 15 ml Coenzyme Diluent to dissolve before use.

Standard:add 1 mldistilled water to dissolve before use; then add 0.2 ml into 0.8 mldistilled 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 mlAssay buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s,repeat 30 times); centrifuged at 16,000g 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 16,000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Warmall reagentsto room temperature before use.

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 µl	--
Distilled water	--	--	200 µl
Sample	10 µl	--	--
Coenzyme	150 µl	--	--
Mix, wait for 5 minutes.			
Substrate	40 µl	--	--
Mix,measured at 340 nm and recordthe absorbance of 10thsecond 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 ADHactivity is the enzyme thatproduces 1nmol of NADH per minute.

1. According to the protein concentration of sample

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

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

2. According to the weight of sample

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

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

3.According to the quantity of cells or bacteria

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

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

4. According to the volume of serum or plasma

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

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

$C_{Standard}$: the standard concentration, 400 μmol/L = 400nmol/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 ×10^4;

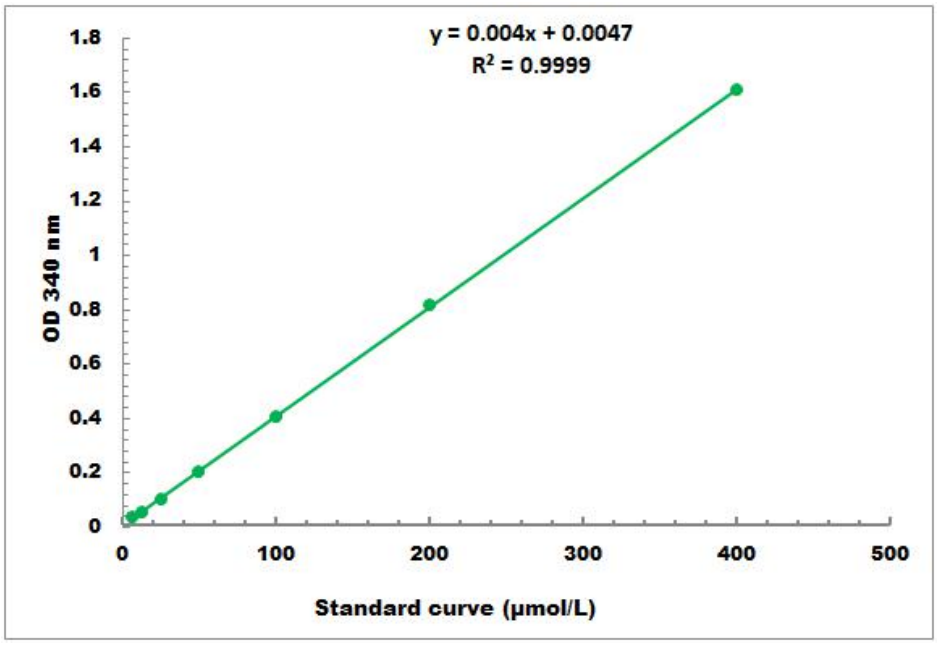
V_{Sample}: the volume of sample, 0.01 ml;

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: 4μmol/L - 400 μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES