



Aldolase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1232

(Version 1.1A)

Detection and Quantification of Aldolase (Ald) Activity in 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

Aldolase (Fructose-Bisphosphate Aldolase: EC 4.1.2.13) is an important enzyme for both glycolysis and gluconeogenesis. It catalyzes the reversible reaction of fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate & dihydroxyacetone.

There are 2 classes of Aldolase - class I: found in animal and plant tissues and class II: found in prokaryotes and lower eukaryotes. Class I Aldolase has 3 isozymes- Type A: found in muscle and red blood cells, Type B: found in liver and kidney and Type C: found in brain. Aldolase A deficiency leads to myopathy & hemolytic anemia. Muscle disease and liver injury can also cause increased serum aldolase. Accurate detection of aldolase activity is valuable for diagnostic and mechanistic studies.

Aldolase Activity Colorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of aldolase activity in a variety of samples. Aldolase converts fructose-1,6-bisphosphate (FPB) to glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone (DHAP). NADH is generated through a linked enzymatic reaction, which reacts with dye reagent to form a colored product. The color intensity increase at 450nm is proportional to Aldolase activity in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	5 ml x 1	4 °C
Diluent	2 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Coenzyme	Powder x 1	-20 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
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

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2 mmol/L of standard stock solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Then dilute to 400 µmol/L standard top solution by adding 0.2 ml stock solution into 0.8 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5/6.25 µmol/L.

Substrate: Briefly centrifuge prior to opening. Add 1 ml Diluent to dissolve before use; store at 4 °C for 3-5 days or -20 °C for 1 month after reconstitution.

Coenzyme: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use. Keep in dark and store at 4°C for 1 week or -20 °C for 2 months.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Diluent to dissolve before use. Keep in dark and store at 4°C for 3-5 days or -20 °C for 1 month after reconstitution.

Dye Reagent A: Add 10 ml distilled water to dissolve before use, mix. 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 200 µl Assay Buffer to dissolve before use; store at -80 °C for 1 month after reconstitution.

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 ml Assay 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 10 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 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detection directly or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Warm the all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Reaction Buffer	50 μ l	50 μ l	50 μ l	--	--
Sample	10 μ l	--	--	--	--
Distilled water	--	10 μ l	--	--	90 μ l
Positive Control	--	--	10 μ l	--	--
Substrate	10 μ l	10 μ l	10 μ l	--	--
Coenzyme	10 μ l	10 μ l	10 μ l	--	--
Enzyme	10 μ l	10 μ l	10 μ l	--	--
Standard	--	--	--	90 μ l	--
Dye Reagent A	100 μ l	100 μ l	100 μ l	100 μ l	100 μ l
Dye Reagent B	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l
Mix, incubate for 5 minutes, measured at 450 nm and record the absorbance.					

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 Aldolase activity is defined as the enzyme generates 1 μmol of NADH per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2 According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

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

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, $\mu\text{mol/mL}$

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

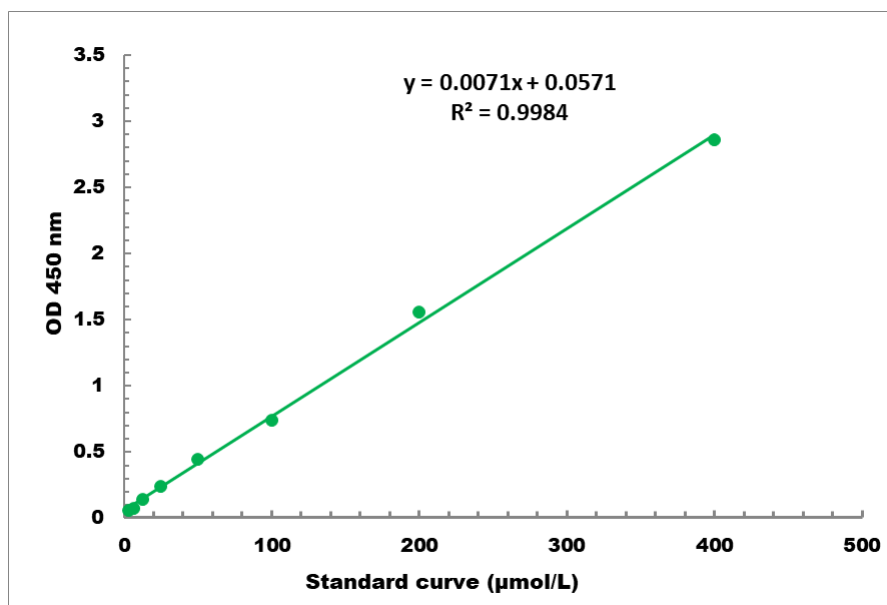
V_{Sample} : the volume of sample in assay procedure, mL

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

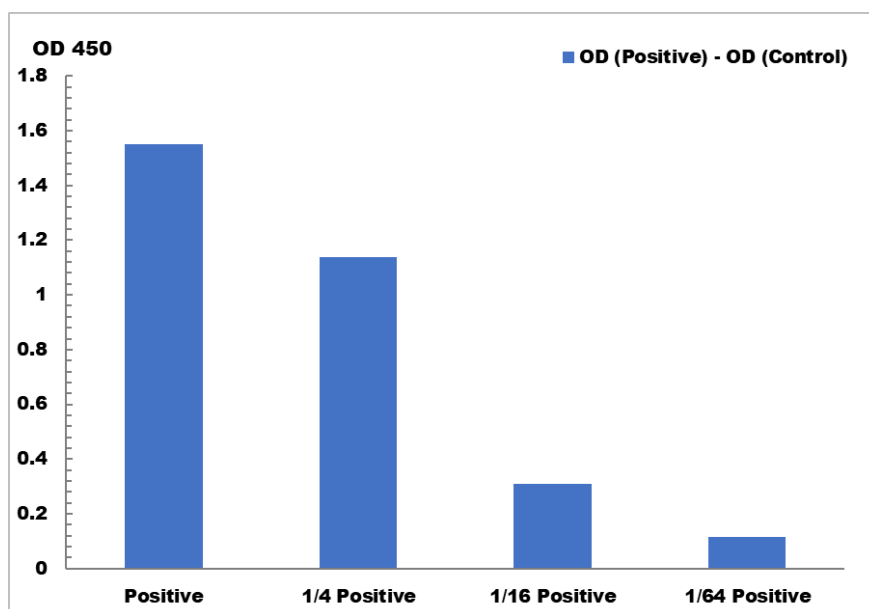
T: the reaction time, minute

VIII. TYPICAL DATA

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



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



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