Glutamate Dehydrogenase Activity Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1066

(Version 1.5E)

Detection and Quantification of Glutamate Dehydrogenase(GDH)Activity in Tissue extracts, Cell lysateSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Glutamate Dehydrogenase (GDH) is a mitochondrial enzyme that catalyzes the reversible oxidative deamination of glutamate to a-ketoglutarate and serves as a key link between anabolic and catabolic pathways. In mammals, GDH is subject to allosteric regulation and has high activity in liver, kidney, brain, and pancreas. GDH activity in serum can be used to differentiate between liver diseases due to liver inflammation, which do not show elevated serum GDH activity, and diseases that result in hepatocyte necrosis, which results in elevated serum GDH.

Glutamate Dehydrogenase ActivityColorimetric Microplate Assay Kit provides a simple and direct procedure for measuring glutamate dehydrogenaseactivity in a variety of samples.The assay is initiated with the enzymatic catalysis of NH_4^+,α ketoglutaric acid and NADH by GDH. NADH can be measured at a colorimetric readout at 450 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Substrate Dilution	20 ml x 1	4 °C
Substrate	Powderx 1	-20 °C
Standard	Powder x 1	-20 °C
Positive Control	Powder x 1	-20 °C
Dye ReagentA	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °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. Centrifuge
7. Timer
8. Ice

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.

Substrate: Add 13 ml Substrate Dilution into Substrate before use, mix. Keep in dark for immediate use or store at -20°C for 2-3weeks after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use, then add 0.5 ml into 0.5 ml distilled water, mix. Store at -80°C for 1 month after reconstitution.

Dye Reagent A: Briefly centrifuge prior to opening. Add 5 ml distilled water to dissolve before use, mix, store at 4°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 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 10minutes, 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
Substrate	130 µl	130 µl	130 µl	--	--
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	--	40 µl	140 µl
Positive Control	--	--	10 µl	--	--
Mix andincubate at room temperature for 5 minutes.					
Standard	--	--	--	100 µl	--
Dye Reagent A	50 µl	50 µl	50 µl	50 µl	50 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl	10 µl
Mix andincubate at room temperature for 2 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.

VII. CALCULATION

Unit Definition: One unit ofGDHactivity is defined as the enzyme that decomposes 1μmol of NADH per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

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

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/L

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

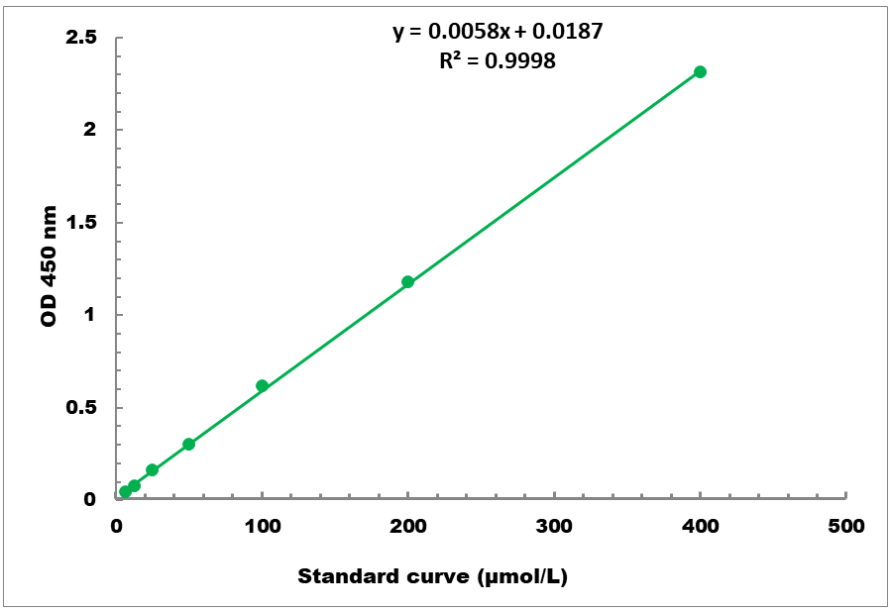
V_{Sample}: the volume of sample in assay procedure, mL

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

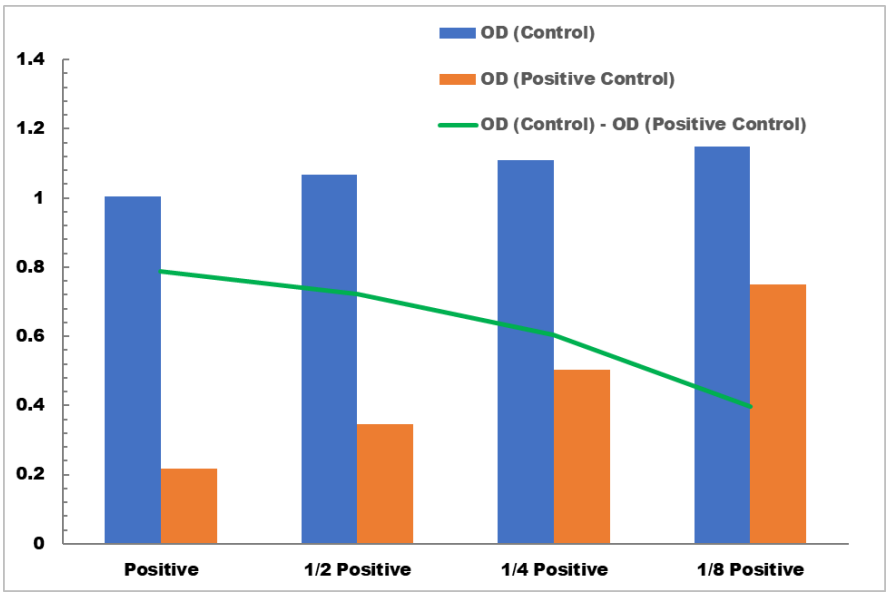
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 4μmol/L - 400 μmol/L



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