6-Phosphogluconate Dehydrogenase Microplate Assay Kit

User Manual

Catalog # CAK1139

(Version 1.3A)

Detection and Quantification of 6-Phosphogluconate Dehydrogenase Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

6-Phosphogluconate dehydrogenase (6PGD) is an enzyme in the pentose phosphate pathway. It forms ribulose 5-phosphate from 6-phosphogluconate.

It is an oxidative carboxylase that catalyses the decarboxylating reduction of 6-phosphogluconate into ribulose 5-phosphate in the presence of NADP. This reaction is a component of the hexose mono-phosphate shunt and pentose phosphate pathways (PPP). Prokaryotic and eukaryotic 6PGD are proteins of about 470 amino acids whose sequences are highly conserved. The protein is a homodimer in which the monomers act independently: each contains a large, mainly alpha-helical domain and a smaller beta-alpha-beta domain, containing a mixed parallel and anti-parallel 6-stranded beta sheet. NADP is bound in a cleft in the small domain, the substrate binding in an adjacent pocket.

The reaction velocity is determined by measuring the increase in absorbance at 340 nm resulting from NADPH.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C
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Note:

Substrate: add 2 ml Assay Buffer to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled 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, multi-channel 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 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.

V. ASSAY PROCEDURE

Warm all regents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 µl	--
Distilled water	--	--	200 µl
Assay Buffer	170 µl	--	--
Substrate	20 µl	--	--
Sample	10 µl	--	--
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

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

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of 6PGD activity is defined as the enzyme produce 1 μmol NADPH per minute.

1. According to the protein concentration of sample

$$6PGD\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 4 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$6PGD\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 4 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$6PGD\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 4 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$6PGD\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 4 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 0.4 μmol/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;

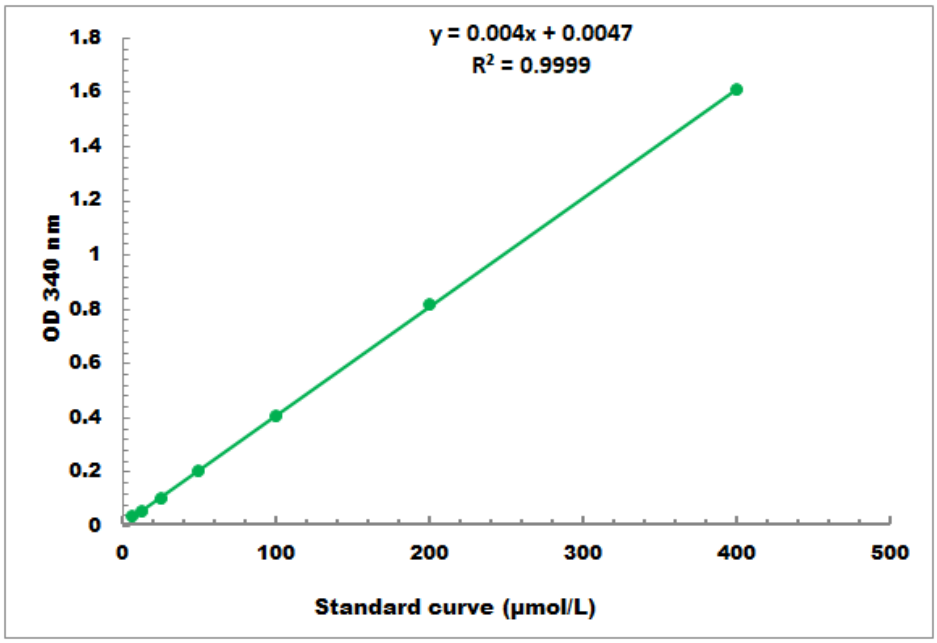
V_{Sample}: the volume of sample, 0.01 ml;

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: 4 μmol/L - 400 μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES