CheKine™ Glucose-6-Phosphate Dehydrogenase (G6PDH) Activity Colorimetric Assay Kit

Cat #: KTB1011 Size: 48 T/96 T

	Glucose-6-Phosphate Dehydrogenase (G6PDH) Activity Colorimetric Assay Kit		
REF	**Cat #:** KTB1011	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant, Bacteria		
	Storage: Stored at 4℃ for 12 months		

Assay Principle

Glucose-6-phosphate dehydrogenase (G6PD or G6PDH) (EC 1.1.1.49) is a cytosolic enzyme that catalyzes the chemical reaction. This enzyme participates in the Pentose Phosphate pathway, a metabolic pathway that supplies reducing energy to cells (such as erythrocytes) by maintaining the level of the co-enzyme Nicotinamide Adenine Dinucleotide Phosphate (NADPH). The NADPH in turn maintains the level of Glutathione in these cells that helps protect the red blood cells against oxidative damage from compounds like hydrogen peroxide. CheKine™ Glucose-6-Phosphate Dehydrogenase (G6PDH) Activity Colorimetric Assay Kit provides a simple method for detecting Glucose-6-Phosphate Dehydrogenase (G6PDH) activity in a variety of biological Samples such as Serum, Plasma, Animal and Plant Tissues, Cells and bacteria. In the assay, G6PDH present in the sample converts $NADP^+$ to NADPH, which has an absorbance at 340 nm. The absorbance of NADPH is proportional to the G6PDH activity present in the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Assay Buffer	60 mL	60 mL×2	4℃
6-Phosphogluconic Acid	1	1	4℃, protected from light
$NADP^+$	1	1	4℃, protected from light

Materials Required but Not Supplied

·Standard microplate reader capable of measuring absorbance at 340 nm
·96-well UV microplate (Cat# BMB0001, Abbkine), Precision pipettes, Disposable pipette tips
·Deionized Water
·Dounce Homogenizer(for Tissue Samples)

Reagent Preparation

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Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

6-Phosphogluconic Acid Working Reagent: Add 2 mL Assay Buffer to dissolve before use. Keep on ice while in use. This solution can be stored at 4℃, protected from light, for up to 1 week.

$NADP^+$ Working Reagent: Add 2 mL Assay Buffer to dissolve before use. Keep on ice while in use. This solution can be stored at 4℃, protected from light, for up to 1 week.

Sample Preparation

1. Animal Tissue samples: Weigh 0.1 g tissue, add 1 mL Assay Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 minutes at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Plant Tissue samples: Weigh 0.1 g tissue, add 1 mL Assay Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 minutes at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Cells or Bacteria: Collect 2 x 10^7 Cells or bacteria into the centrifuge tube, wash Cells or bacteria with cold PBS, discard the supernatant after centrifuge at 12,000 g for 1 minutes at 4℃， add 1 mL Assay Buffer to ultrasonically disrupt the cells or bacteria on ice for 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 minutes at 4℃. Use supernatant for assay, and place it on ice to be tested.
4. Plasma, Serum and other Liquid Samples: Tested directly by adding samples to the microplate.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine catalog number: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheated the microplate reader for more than 30 min, and adjust the wavelength to 340 nm.
2. Preheated Assay Buffer for more than 30 min at 25℃ or 37℃ bath.
3. Add materials into each well (use 96-well UV plate) as below:

Regent	Blank well (μL)	Test well (μL)
Sample	0	20
Deionized Water	20	0
Assay Buffer	140	140
6-Phosphogluconic Acid Working Reagent	20	20
$NADP^+$ Working Reagent	20	20

Mix well and Monitor absorbance at 340 nm within 3 min. For Blank well, measure OD340 nm at 10 s to read A_1 and 190 s to read A_2, calculate $\triangle A_{Blank}=A_2-A_1$. For Test well, measure OD340 nm at 10 s to read A_3 and 190 s to read A_4, calculate $\triangle A_{Test}=A_4-A_3$.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Calculation formulae based on 96-well UV plates are as below:

1. Calculation by volume of liquid

Unit Definition: One unit defines as the amount of enzyme that catalyzes and generates 1.0 μmol NADPH per mL of liquid per minute.

G6PDH Activity (U/mg prot)=$[(\Delta A_{Test}-\Delta A_{Blank})\times V_{Total}\div(\varepsilon\times d)\times 10^6]\div V_{Test}\div T$**$=1.0718\times(\Delta A_{Test}-\Delta A_{Blank})$**

2. Calculated by protein concentration

Unit Definition: One unit defines as the amount of enzyme that catalyzes and generates 1.0 μmol NADPH per milligram of protein per minute.

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G6PDH Activity (U/mg prot)=[(ΔA_{Test} -ΔA_{Blank})×V_{Total}÷(ε×d)×10^6]÷(Cpr×V_{Test})÷T**=1.0718×(ΔA_{Test} - ΔA_{Blank})÷Cpr**

3. Calculated by fresh weight of samples

Unit Definition: One unit defines as the amount of enzyme that catalyzes and generates 1.0 μmol NADPH per gram of sample per minute.

G6PDH Activity (U/g)=[(ΔA_{Test}-ΔA_{Blank})×V_{Total}÷(ε×d)×10^6]÷(V_{Test}÷$V_{Extract}$×W)÷T**=1.0718×(ΔA_{Test} - ΔA_{Blank})÷W**

4. Calculated by Cells or Bacteria density

Unit Definition: One unit defines as the amount of enzyme that catalyzes and generates 1.0 μmol NADPH per 10^4 cell of sample per minute.

G6PDH Activity (μmol/min/10^4 Cells or Bacteria)=[(ΔA_{Test}-ΔA_{Blank})×V_{Total}÷(ε×d)×10^6]÷(2000×V_{Test}÷$V_{Extract}$)÷T**=0.0005359×(ΔA_{Test} - ΔA_{Blank})**

Where:$\Delta A_{Test}=A_4-A_3$; $\Delta A_{Blank}=A_2-A_1$; ε: The extinction coefficient of NADPH, 6.22×10^3 L/mol/cm; d: The optical path of 96 well UV plate, 0.5 cm; 10^6: 1 mol=1×10^6 μmol; V_{Test}: volume of sample added to the reaction system, 20 μL= 0.02 mL; V_{Total}: The total volume of reaction system, 0.0002 L; Cpr: Protein concentration of the sample, mg/mL; $V_{Extract}$: Volume of Assay Buffer, 1 mL; W: Fresh weight of samples, g; T: Reaction time, 3 min; 2000: number of Cells, 2×10^7.

Recommended Products

Catalog No.	Product Name
KTB1010	CheKine™ NADP/NADPH Assay Kit
KTB1600	CheKine™ Reduced Glutathione (GSH) Colorimetric Assay Kit
KTB1610	CheKine™ Glutathione Oxidized (GSSG) Colorimetric Assay Kit

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