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

Cat #: KTB1011 Size: 48 T/48 S 96 T/96 S

	Micro Glucose-6-Phosphate Dehydrogenase (G6PDH) Activity 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, protected from light		

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™ Micro Glucose-6-Phosphate Dehydrogenase (G6PDH) Activity 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	70 mL	70 mL×2	4℃
6-Phosphogluconic Acid	Powder×1 vial	Powder×1 vial	4℃, protected from light
$NADP^+$	Powder×1 vial	Powder×1 vial	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Standard microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Dounce homogenizer (for tissue samples)

Reagent Preparation

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

6-Phosphogluconic Acid Working Reagent: Add 2.4 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 2.4 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 min 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 min 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 min 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 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
4. Plasma, serum and other liquid samples: Tested directly.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheated the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
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 or microquartz cuvette) as below:

Reagent	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

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

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.002, increase the sample quantity appropriately. If ΔA_{Test} is larger than 0.6, the sample can be appropriately diluted with Assay Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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.

A. 96-well UV plate calculation formula

1. Calculation by volume of liquid

Unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1 μmol NADPH per mL of liquid per min.

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 μmol NADPH per mg of protein per min.

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

3. Calculated by fresh weight of samples

Unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1 μmol NADPH per g of sample per min.

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

4. Calculated by cells or bacteria density

Unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1 μmol NADPH per 10^4 cells or bacteria per min.

G6PDH Activity (μmol/min/10^4)=$[(\Delta A_{Test}-\Delta A_{Blank})\times V_{Total}\div(\varepsilon\times d)\times 10^6]\div(2000\times V_{Test}\div V_{Extract})\div T$**$=0.0005359\times(\Delta A_{Test}-\Delta A_{Blank})$**

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; 2,000: number of cells or bacteria, 2×10^7.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Recommended Products

Catalog No.	Product Name
KTB1010	CheKine™ Micro Coenzyme II NADP(H) Assay Kit
KTB1600	CheKine™ Micro Reduced Glutathione (GSH) Assay Kit
KTB1610	CheKine™ Micro Glutathione Oxidized (GSSG) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.