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Glucose-6-phosphate dehydrogenase (G6PDH) kit (visible colorimetric method), Microplate method

Product Number: G6PDH002

Shipping and Storage

Valid for 3 months, different reagents have different storage temperatures, please refer to the actual reagent label for storage.

Component

Component	96T	Storage
Extraction Buffer	100mL × 1	4℃
Reagent 1	25mL × 1	4℃
Reagent 2	Powder, 1 bottle	4℃
Reagent 3	1.1mL × EP tube	4℃, protected from light
Standard	Powder, 1 vial	-20℃

Note: Reagent 2: Tap the bottle gently to settle all powder to the bottom before opening; Add 19mL of Reagent 1 to dissolve thoroughly. Unused reagent shall be stored at 4°C.

Standard: This reagent is used when a new standard curve needs to be prepared; Prepare according to the standard curve preparation steps in the instruction; Use up the dissolved standard within one week.

Description

Glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) is widely present in animals, plants, microorganisms and cultured cells. It is a key enzyme in the pentose phosphate pathway, and maintains the intracellular level of NADPH. NADPH in turn maintains the glutathione level in cells, thereby protecting cells from oxidative damage. Therefore, the activity of G6PDH can reflect the biosynthesis and antioxidant capacity of organisms to a certain extent.

G6PDH catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconolactone, and simultaneously reduces $NADP^+$ to NADPH. The traditional method detects the absorbance of NADPH at 340nm. Due to the low molar extinction coefficient (ε) of NADPH, this method has low sensitivity and is severely susceptible to interference.

This kit provides a simple, sensitive and rapid assay method: the NADPH produced by this enzymatic process reacts with a specific chromogenic reagent to produce a colored substance with a maximum absorption peak at 450nm. The G6PDH enzyme activity is calculated by detecting the increase rate of absorbance at 450nm.

Experimental Equipment

Mortar (homogenizer), ice box (ice maker), bench-top centrifuge, adjustable pipette, water bath (oven, incubator, dry bath), 1mL cuvette, centrifuge tubes, spectrophotometer, distilled water (deionized water or ultrapure water is acceptable).

Assay Procedure

It is recommended to select 1–3 samples with large differences (e.g., different types or groups) for a pre-experiment to familiarize with the operation process. Determine or adjust the sample concentration according to the pre-experiment results to avoid unnecessary waste of samples or reagents!

Sample Extraction

1. Tissue samples: Weigh approximately 0.1 g of tissue, add 1mL of extraction buffer, and homogenize in an ice bath. Centrifuge at 12,000 rpm and 4°C for 15 min, collect the supernatant and place on ice for assay.

Note: If the sample amount is increased, extraction can be performed at a ratio of tissue mass (g) : extraction buffer volume (mL)

= 1 : 5–10.

2. Bacteria/cell samples: Collect bacteria or cells into centrifuge tubes first, and discard the supernatant after centrifugation. Take 5 million bacteria or cells and add 1mL of extraction buffer. Sonicate to disrupt bacteria or cells (ice bath, power 20% or 200 W, sonication for 3 s, interval for 10 s, repeat 30 times). Centrifuge at approximately 12,000 rpm and 4°C for 10 min, and take the supernatant as the test sample.

   Note: If the sample amount is increased, extraction can be performed at a ratio of bacteria/cell count (10^4) : extraction buffer (mL) = 500–1000 : 1. In this experiment, lysis buffer can also be used to lyse cell samples for subsequent detection, and the lysis method shall follow the operation instructions of the lysis buffer.

3. Liquid samples: Detect directly. If turbid, centrifuge and take the supernatant for detection.

Protocol

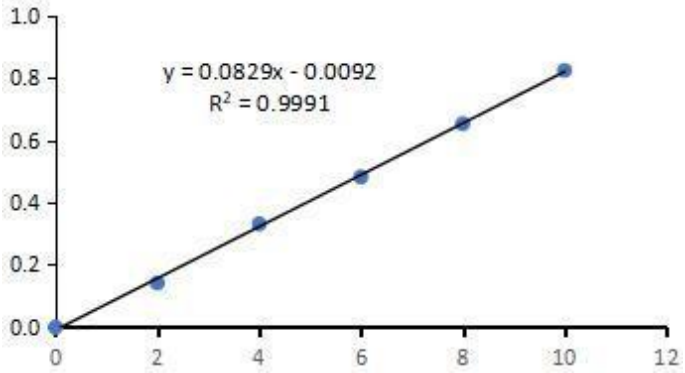
1. Preheat the spectrophotometer for more than 30 min, set the temperature to 37°C, adjust the wavelength to 450nm.
2. Incubate the reagents in a 37°C water bath for 5–15 min. Add the following reagents sequentially into a 1mL glass cuvette:

Component	Assay Tube
Sample	10μL
Reagent 2	180μL
Reagent 3	10μL
Mix thoroughly, immediately read the A1 value at 450nm. Incubate at 37°C for 20 min and read the A2 value (Observation: the higher the enzyme activity, the more obvious the yellow color). ΔA = A2 −A1.	

Note: If ΔA is too small, the reaction time can be extended (e.g., 60 min or longer) before reading A_2, or the sample volume can be increased (e.g., to 0.2 g). The adjusted reaction time T shall be substituted into the calculation formula for recalculation.

Result Calculation

1. Standard curve equation:y =0.0829x-0.0092, where x is the molar amount of NADPH (nmol), and y is ΔA.



2. Calculation based on sample protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1nmol of glucose-6-phosphate to 1nmol of 6-phosphogluconolactone and converts 1nmol of $NADP^+$to 1nmol of NADPH per minute per milligram of tissue protein at 37°C.

G6PDH(nmol/min /mg prot)=[(ΔA+0.0092)÷0.0829]÷ (V1×Cpr) ÷T=60.314×(ΔA+0.0092)÷Cpr

3. Calculation based on sample fresh weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1 nmol of glucose-6-phosphate to 1 nmol of 6-phosphogluconolactone and converts 1 nmol of $NADP^+$ to 1 nmol of NADPH per minute per gram of tissue at 37°C.

G6PDH(nmol /min /g 鲜重)=[(ΔA+0.0092)÷0.0829]÷(W×V1÷V) ÷T=60.314×(ΔA+0.0092)÷W

4. Calculation based on cell count:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1 nmol of glucose-6-phosphate to

1 nmol of 6-phosphogluconolactone and converts 1 nmol of $NADP^+$ to 1 nmol of NADPH per minute per 10^4 cells at 37°C.

G6PDH(nmol /min /104 cell)=[(ΔA+0.0092)÷0.0829]÷(500×V1÷V)÷T=0.1206×(△A+0.0092)

5. **Calculation of G6PDH activity in liquid samples:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1nmol of glucose-6-phosphate to 1nmol of 6-phosphogluconolactone and converts 1nmol of $NADP^+$ to 1nmol of NADPH per minute per milliliter of liquid at 37°C.

G6PDH(nmol/min /mL)=[(ΔA+0.0092)÷0.0829]÷V1÷T=60.314×(ΔA+0.0092)

V — volume of added extraction buffer, 1mL;

V1— volume of added sample, 0.01mL;

W — sample mass, g;

T — reaction time, 20 min; if the reaction time is extended, the adjusted reaction time value shall be substituted into the formula for recalculation;

Cpr — sample protein concentration, mg/mL; it is recommended to use the BCA Protein Assay Kit of our company.

Appendix: Standard Curve Preparation Procedure

1. Add 0.6mL of distilled water into the standard EP tube (the stock solution shall be used within two days and stored at -20°C). The concentration of the standard stock solution is 1nmol/μL. Dilute the stock solution with distilled water into six concentration gradients, e.g., 0, 0.2, 0.4, 0.6, 0.8, 1nmol/μL. The standard concentration can also be adjusted according to actual samples.
2. Reference table for standard dilution:

Standard concentration	0	0.2nmol/μL	0.4nmol/μL	0.6nmol/μL	0.8nmol/μL	1nmol/μL
Standard diluent	0	40μL	80μL	120μL	160μL	200μL
Water	200μL	160μL	120μL	80μL	40μL	0
Mix all standard tubes well for later use.						

3. Operate according to the sample addition table. Based on the results, subtract the absorbance of 0 concentration from the absorbance of each concentration, and plot the standard curve passing through the zero point.

Component	Standard Tube	0 Concentration Tube (performed only once)
Standard	10μL	-
Distilled water	-	10μL
Reagent 1	180μL	180μL
Reagent 3	10μL	10μL
Mix well, let stand at room temperature for 5 min, then read the value at 450nm. Δ A = A_{assay} − A_{0 concentration tube}.		