Glucose-6-Phosphate（P6G）Content Assay Kit

Detection Equipment: Spectrophotometer/Microplate Reader
Catalog Number: BC5965
Size: 100T/96S

Components:Please carefully check the volume of the reagent and the volume in the bottle before use. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation condition
Extract	Liquid 110 mL×1	2-8℃ storage
Reagent I A	Powder ×1	2-8℃ storage
Reagent I B	Liquid 45 mL×1	2-8℃ storage
Reagent II	Powder ×1	2-8℃ storage
Reagent III	Powder ×1	-20℃ storage
Standard	Powder ×1	2-8℃ storage

Solution reparation :

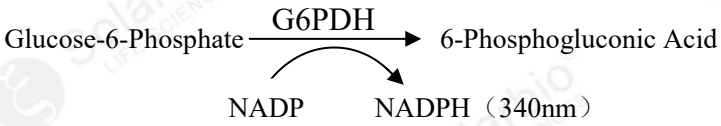
1. Reagent I: Before the use of Reagent A all dissolved in Reagent B, fully dissolved after use, the unused reagent 2-8℃ storage for 4 weeks (reagent color from transparent to light yellow is normal, can be used normally), prohibited to put -20℃ storage.
2. Reagent II: Before use, add 0.5mL distilled water to fully dissolve, and storage the unused reagent for 4 weeks at -20℃ to avoid repeated freezing and thawing.
3. Reagent III: Before use, add 1mL distilled water to fully dissolve, unspent reagent -20℃ sub-storage for 4 weeks, to avoid repeated freeze-thaw (the reagent is a freeze-dried reagent, there may be a large difference or even a small amount of visual observation of the dose in different bottles, this phenomenon does not affect the use, the actual quality is the same).
4. Reagent III working liquid: before clinical use according to the sample size according to Reagent III: distilled water =3μL: 12μL (a total of 15μL, about 4T) the proportion of preparation and use, now used.
5. Standard: Before use, add 1.645mL distilled water to fully dissolve into 20μmol/mL glucose 6-phosphate standard, storage at 2-8℃ for 4 weeks.
6. Preparation of 0.5μmol/mL standard: Take 25μL 20μmol/mL standard of glucose 6-phosphate and add 975μL distilled water to fully dissolve it into 0.5μmol/mL standard for determination, and then use it now.
7. Working liquid: Before clinical use, according to the sample size, Reagent I: Reagent II: Reagent III working liquid =870μL: 15μL: 15μL (a total of 900μL, about 4T) is prepared into working liquid and used now.

Description:

Glucose-6-Phosphate (G6P), also known as glucose-6-phosphate, is an intermediate product of glycolysis and pentose phosphate pathway, which is widely found in animals, plants and microorganisms. In the first step of glycolysis, glucose is catalyzed by hexokinase to form glucose-6-phosphate, which is then catalyzed by phosphate glucose isomerase to form

fructose-6-phosphate to continue the other steps of glycolysis. However, in the pentose phosphate pathway, glucose-6-phosphate is its first substrate, and this process is also the main way to generate NADPH. "In addition, glucose-6-phosphate can also be converted to glycogen or starch and stored.

Glucose-6-phosphate dehydrogenase catalyzes the formation of glucose-6-phosphate acid and NADPH from G-6-P and NADP+, with an absorption peak at 340nm, from which the content of glucose-6-phosphate can be calculated.



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

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate Reader, micro glass cuvette/96 well flat-bottom plate, balance, cryogenic centrifuge, water bath/thermostatic incubator, mortar/homogenizer, ice and distilled water.

Protocol:

I. Sample processing (The sample size to be tested can be appropriately adjusted, and the specific proportion can be referred to the literature)

Tissue: According to the ratio of mass (g) : volume of Extraction (mL)=1:5-10 (it is recommended to weigh about 0.1g and add 1mL of Extraction), add the Extraction, homogenize in an ice bath, boil in a boiling water bath for 5min (seal with a sealing membrane to prevent the cap from breaking), cool to room temperature, centrifuged at 12000g for 10min at 4℃, and take the supernatant to be tested on ice.

II. Measurement Steps

1. The ultraviolet spectrophotometer was preheated for more than 30min, the wavelength was adjusted to 340nm, and the distilled water was zeroed.
2. Working fluid at 37℃ for 5min.
3. Sample determination (Add the following reagents to micro glass cuvette/96 well flat-bottom plate)

Reagent name（μL）	Test tube	Standard tube	Blank tube
Sample	75	-	-
Standard	-	75	-
Distilled water	-	-	75
Working liquid	225	225	225
Thoroughly mixed, immediately determine the absorbance A1 at 10s at 340nm, and then quickly react at 37℃ for 10min to determine the absorbance A2 at 10min10s, respectively recorded as $A1_{text}$, $A2_{text}$, $A1_{standard}$, $A2_{standard}$, $A1_{blank}$, $A2_{blank}$. Calculate $\Delta A_{standard} = (A2_{standard} - A1_{standard}) - (A2_{blank} - A1_{blank})$, $\Delta A_{text} = (A2_{text} - A1_{text}) - (A2_{blank} - A1_{blank})$. Each standard tube and blank tube only need to be tested 1-2 times.			

III. Calculations

1. Calculated by sample protein concentration:

G6P content（μmol/g prot）=ΔA_{text}÷（$\Delta A_{standard}$÷$C_{standard}$）×$V_{extraction}$÷（Cpr×$V_{extraction}$）×F

=0.5×ΔA_{text}÷$\Delta A_{standard}$÷Cpr×F

2. Calculated by sample quality:

G6P content（μmol/g mass）=ΔA_{text}÷（$\Delta A_{standard}$÷$C_{standard}$）×$V_{extraction}$÷W×F

=0.5×ΔA_{text}÷$\Delta A_{standard}$÷W×F

C standard: standard point concentration, 0.5μmol/mL;

V extraction: add the volume of extract solution, 1mL;

Cpr: sample protein concentration, mg/mL;

W: sample quality, g;

F: dilution factor.

Note:

1. It is best for two people to do this experiment at the same time, one person to compare colors and one person to time, in order to ensure the accuracy of the experimental results.
2. If the sample ΔA<0.01, the sample size can be appropriately increased after determination; If the sample ΔA>1.0 or A text>1.5, can be measured after diluting the supernatant with distilled water. Note that the dilution factor in the calculation formula is changed simultaneously.

Experimental example:

1. Take 0.102g rat liver and add it into the extract for ice bath homogenization. After taking the supernant, follow the determination procedure and use 96-well UV plate to measure ΔA = ($A2_{text}$-$A1_{text}$) - ($A2_{blank}$ -$A1_{blank}$) =0.281-0.177）-（0.045-0.045）=0.104. $\Delta A_{standard}$ = ($A2_{standard}$ -$A1_{standard}$) - ($A2_{blank}$ -$A1_{blank}$) =（0.470-0.074）-（0.045-0.045）=0.396. The content of glucose 6-phosphate calculated according to the sample quality is:
   G6P content (μmol/g mass) =0.5×ΔA_{text}÷$\Delta A_{standard}$ ÷W×F =1.287 μmol/g mass.
2. Take 0.103g of rat lung was added into the extract and homogenized in an ice bath, and the supernatant was removed and follow the determination procedure and use 96-well UV plate to measure ΔA = ($A2_{text}$-$A1_{text}$) - ($A2_{blank}$ -$A1_{blank}$) =（0.179-0.113）-（0.045-0.045）=0.066. $\Delta A_{standard}$ = ($A2_{standard}$ -$A1_{standard}$) - ($A2_{blank}$ -$A1_{blank}$) =（0.470-0.074）-（0.045-0.045）=0.396. The content of glucose 6-phosphate calculated according to the sample quality is:
   G6P content (μmol/g mass) =0.5×ΔA_{text}÷$\Delta A_{standard}$ ÷W×F =0.809 μmol/g mass.

References：

[1] Belford J, Feinleib M R .The increase in glucose-6-phosphate content of the heart after the administration of inotropic catecholamines, calcium, and aminophylline[J].Biochemical Pharmacology, 1962, 11(11):987-994.

[2] Ashcroft S , Capito K , Hedeskov C .Time course studies of glucose-induced changes in glucose-6-phosphate and fructose-1, 6-diphosphate content of mouse and rat pancreatic islets[J].Diabetologia, 1973, 9(4):299-302.

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