Fructokinase(FRK) Activity Assay Kit

Detection Equipment: Spectrophotometer/microplate reader

Catalog Number: BC5925

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
Powder I	Powder ×1	2-8℃ storage
Reagent I	Powder ×1	-20℃ storage
Reagent II	Powder ×1	-20℃ storage
Reagent III	Liquid 3 mL×1	2-8℃ storage
Reagent IV	Liquid 12 mL×1	2-8℃ storage
Reagent V	Liquid 8μL×2	-20℃ storage
Reagent VI	Powder ×2	-20℃ storage

Solution reparation :

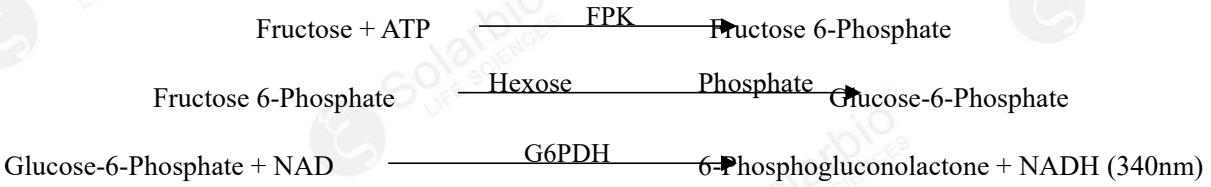
1. Extraction solution: Dissolve the powder in the extraction solution before use; The reagent is a suspension that can be shaken well before use and storage at 2-8℃ for 12 weeks;
2. Reagent I: The reagent is placed in the glass tube in the reagent bottle. Before use, add 2.667mL distilled water and dissolve the dissolved reagent fully. The dissolved reagent is storage at -20℃ for 4 weeks to avoid repeated freezing and thawing.
3. Reagent II: The reagent is placed in the glass tube in the reagent bottle. Before use, add 3.03mL distilled water and dissolve the dissolved reagent fully. The dissolved reagent is storage at -20℃ for 4 weeks to avoid repeated freezing and thawing.
4. Reagent III: Put the reagent in the glass bottle of the reagent bottle, add 6mL distilled water to dissolve before use, and storage the dissolved reagent in 2-8℃ for 4 weeks;
5. Reagent V Working liquid: Before clinical use according to the number of samples in accordance with Reagent V: distilled water =8μL: 1000μL (a total of 1008μL, about 100T) the proportion of preparation, fully mixed, ready for use;In order to prolong the use of this product, give one more.
6. Reagent VI: Before use, take a Reagent VI and add 1mL distilled water to dissolve it fully. The dissolved reagent is storage at -20℃ for 2 weeks to avoid repeated freezing and thawing. (This reagent is a freeze-dried reagent, there may be a large difference or even a small amount of macroscopic observation between different bottles, this phenomenon does not affect the use, the actual quality is the same)

Description:

Fructokinase (FRK, EC 2.7.1.4) is a major enzyme that catalyzes fructose phosphorylation. Fructokinase can be used as a hexose receptor and signal molecule in plants to regulate plant metabolism and growth and development by affecting plant growth cycle. Fructose phosphorylation

is important for maintaining the direction of starch biosynthesis.

FRK catalyzes the synthesis of fructose 6-phosphate, which is isomerized to glucose 6-phosphate under the action of hexose phosphate isomerase, and glucose 6-phosphate dehydrogenase further catalyzes the dehydrogenation of glucose 6-phosphate to NADH, which has a characteristic absorption peak at 340nm.



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, low temperature centrifuge, analytical balance, water bath/constant temperature incubator, micro glass cuvette/96 well flat-bottom plate, adjustable pipette, mortar/homogenizer/cell ultrasonic crusher, 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)

1. **Tissue sample:** Add the extract solution according to the ratio of sample mass (g) : extraction liquid volume (mL) =1:5-10 (it is recommended to weigh 0.1g sample and add 1.0mL extraction solution). After homogenization in an ice bath, centrifuge at 8000g at 4℃ for 10min, discard the precipitate, and take the supernatant and place it on ice for testing.
2. **Cell sample:** By cells number (10^6) : Extract liquid volume (mL) =5~ 10:1 ratio to add extract solution (1.0mL extract solution is recommended for 5 million cells), ice bath ultrasonic crushing cells (power 200W, ultrasonic 3s, interval 7s, total time 3min), then centrifuge at 4℃, 8000g, 10min, discard precipitation, take superserum placed on ice to be measured.
3. **Liquid sample:** Direct measurement. If the liquid was cloudy, the supernatant was centrifuged for determination.

II. Measurement Steps

1. The Spectrophotometer/Microplate Reader was preheated for more than 30min, the wavelength was adjusted to 340nm, and the distilled water was zeroed.
2. Preheat Reagent IV at 37℃ for 15min.
3. Add samples in the microquartz colorimetric dish / 96-well UV plate in the following order:

Reagent name（μL）	Test tube	Blank tube
Reagent I	20	20
Reagent II	20	20
Reagent III	20	20
Reagent IV	100	100
Reagent V Working liquid	10	10

Reagent VI	10	10
Distilled water	-	20
Sample	20	-

Immediately and thoroughly mixed, the light absorption value A1 at 340nm was measured for 10s, and was quickly placed in a 37℃ water bath or constant temperature incubator for 5min(The enzyme marker has the temperature control function to adjust the temperature to 37℃). Then, the light absorption value A2 at 5min10s was measured .Calculate $\Delta A_{text}=A2_{text}-A1_{text}$, $\Delta A_{blank}=A2_{blank}-A1_{blank}$, $\Delta A=\Delta A_{text}-\Delta A_{blank}$. Blank only needs to be done 1-2 times.

Note: If the number of samples is large, Reagent I, II, III, IV, V and VI can be proportionally mixed into working liquid for use.

III. Calculations

1. Microquartz colorimeter

1) Calculated by sample protein concentration

Definition of unit: The catalytic production of 1nmol of NADH per mg of hiprotein per minute at 37℃ is defined as one unit of enzyme activity.

FRK activity（U/mg prot）$=\Delta A\div(\varepsilon\times d)\times V_{total\ reaction}\times10^9\div(Cpr\times V_{sample})\div T=321.54\times\Delta A\div Cpr$

2) Calculated by sample quality

Definition of unit:The catalytic production of 1 nmol of NADH per g tissue per minute at 37℃is defined as a unit of enzyme activity.

FRK activity（U/g mass）$=\Delta A\div(\varepsilon\times d)\times V_{total\ reaction}\times10^9\div(W\div V_{extraction}\times V_{sample})\div T=321.54\times\Delta A\div W$

3) Calculated by the number of cells

Definition of unit:The catalytic production of 1 nmol of NADH per 10^6 cells per minute at 37℃is defined as one unit of enzyme activity.

FRK activity（U/10^6 cell）$=\Delta A\div(\varepsilon\times d)\times V_{total\ reaction}\times10^9\div(N\div V_{extraction}\times V_{sample})\div T=321.54\times\Delta A\div N$

4) Calculated by liquid volume

Definition of unit:The catalytic production of 1 nmol of NADH per milliliter per minute at 37℃is defined as a unit of enzyme activity.

FRK activity（U/mL）$=\Delta A\div(\varepsilon\times d)\times V_{total\ reaction}\times10^9\div V_{sample}\div T=321.54\times\Delta A$

ε : NADH molar extinction coefficient, 6.22×10^3 L/mol /cm;

d: Light diameter of 1mL quartz colorimetric dish, 1cm;

$V_{total\ reaction}$: total volume of reaction, 0.2×10^{-3}L;

10^9: Unit conversion, 1mol=10^9nmol;

V_{sample}: Add sample volume, 0.02mL;

$V_{extraction}$: extraction liquid volume, 1mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample quality, g;

N: Number of cells, measured in 10^6;

T: Reaction time, 5min.

2. 96-well UV plate

Change the d-1cm in the above formula to d-0.6cm (96-well UV plate light diameter) for calculation.

Note:

1. If ΔA is less than 0.005, the sample size can be increased or the reaction time can be extended before determination; If the ΔA determination is greater than 0.6 or the A1 determination is less than A1 blank, it is recommended to dilute the sample or shorten the reaction time before the determination. Change the calculation formula simultaneously.

Experimental example:

1. Take 0.1023g potato tissue sample, add the extraction solution for ice bath homogenization, centrifuge the supernatant, follow the measurement procedure, and use microquartz colorimeter dish to measure and calculate: $\Delta A_{text}=A2_{text}-A1_{text}=0.137-0.080=0.057$, $\Delta A_{blank}=A2_{blank}-A1_{blank}=0.001-0.001=0$, $\Delta A=\Delta A_{text}-\Delta A_{blank}=0.057$. Calculated by sample quality:
   FRK activity (U/g mass) =321.54×ΔA÷W = 179.157 U/g mass.
2. Take 0.06g yeast powder, add the extract into the ice bath homogenate, centrifuge and take the supernatant, follow the measurement procedure, and use microquartz colorimeter dish to measure and calculate: $\Delta A_{text}=A2_{text}-A1_{text}=0.705-0.202=0.503$, $\Delta A_{blank}=A2_{blank}-A1_{blank}=0.001-0.001=0$, $\Delta A=\Delta A_{text}-\Delta A_{blank}=0.503$. Calculated by sample quality:
   FRK activity (U/g mass) =321.54×ΔA÷W =2695.58 U/g mass.

References：

[1] Giroix M H, Jijakli H, Courtois P, et al. Fructokinase activity in rat liver, ileum, parotid gland, pancreas, pancreatic islet, B and non-B islet cell homogenates.[J].International Journal of Molecular Medicine, 2006, 17(3):517-522.

[2] Kurt, Bergbauer, Ralf, et al. Studies on Fructose Metabolism in Cultured Astroglial Cells and Control Hepatocytes: Lack of Fructokinase Activity and Imrrunoreactivity in Astrocytes[J].Developmental Neuroscience, 2009, 18(5-6):371-379.

[3] Schaffer A A, Petreikov M. Inhibition of fructokinase and sucrose synthase by cytosolic levels of fructose in young tomato fruit undergoing transient starch synthesis[J].Physiologia Plantarum, 2010, 101(4):800-806.

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