

# Fructokinase(FRK) Activity Assay Kit

**Detection Equipment:** Spectrophotometer

**Catalog Number:** BC5920

**Size:** 50T/48S

**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 60 mL×1	2-8°C storage
Powder I	Powder ×1	2-8°C storage
Reagent I	Powder ×1	-20°C storage
Reagent II	Powder ×1	-20°C storage
Reagent III	Powder ×1	2-8°C storage
Reagent IV	Liquid 30 mL×1	2-8°C storage
Reagent V	Liquid ×3	-20°C storage
Reagent VI	Powder ×3	-20°C storage

**Solution preparation :**

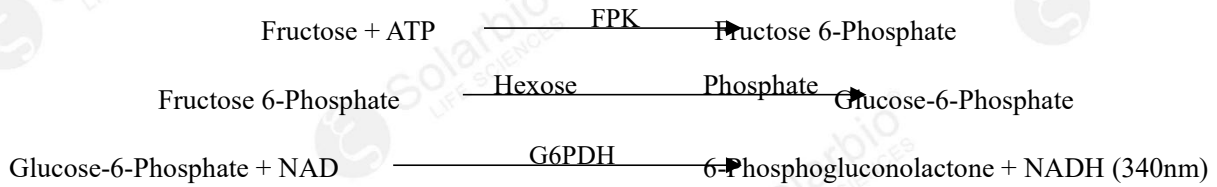
1. Extraction solution: Dissolve the powder in the extraction solution before use; The reagent is a suspension that can be shaken well before use and storage at 2-8°C for 12 weeks;
2. Reagent I: The reagent is placed in the glass tube in the reagent bottle. Before use, add 6mL distilled water and dissolve the dissolved reagent fully. The dissolved reagent is storage at -20°C 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 6mL distilled water and dissolve the dissolved reagent fully. The dissolved reagent is storage at -20°C 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°C 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 20T) the proportion of preparation, fully mixed, ready for use;
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°C 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, low temperature centrifuge, analytical balance, water bath/constant temperature incubator, 1mL quartz colorimetric dish, 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°C 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°C, 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

- The ultraviolet spectrophotometer was preheated for more than 30min, the wavelength was adjusted to 340nm, and the distilled water was zeroed.
- Preheat Reagent IV at 37°C for 15min.
- Add samples in 1mL quartz colorimetric dish in the following order:

Reagent name (μL)	Test tube	Blank tube
Reagent I	100	100
Reagent II	100	100
Reagent III	100	100
Reagent IV	500	500
Reagent V Working liquid	50	50

Reagent VI	50	50
Distilled water	-	100
Sample	100	-

Immediately and thoroughly mixed, the light absorption value A1 at 340nm was measured for 10s, and was quickly placed in a 37°C water bath or constant temperature incubator for 5min. Then, the light absorption value A2 at 5min10s was measured and the light absorption value A1 at 10s at 340nm and the light absorption value A2 after 5min were recorded. Calculate  $\Delta A_{\text{text}} = A2_{\text{text}} - A1_{\text{text}}$ ,  $\Delta A_{\text{blank}} = A2_{\text{blank}} - A1_{\text{blank}}$ ,  $\Delta A = \Delta A_{\text{text}} - \Delta A_{\text{blank}}$ . 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. Calculated by sample protein concentration

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

$$\text{FRK activity (U/mg prot)} = \Delta A \div (\epsilon \times d) \times V_{\text{total reaction}} \times 10^9 \div (\text{Cpr} \times V_{\text{sample}}) \div T = 321.54 \times \Delta A \div \text{Cpr}$$

#### 2. Calculated by sample quality

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

$$\text{FRK activity (U/g mass)} = \Delta A \div (\epsilon \times d) \times V_{\text{total reaction}} \times 10^9 \div (W \div V_{\text{extraction}} \times V_{\text{sample}}) \div T = 321.54 \times \Delta A \div W$$

#### 3. Calculated by the number of cells

Definition of unit: The catalytic production of 1 nmol of NADH per 10<sup>6</sup> cells per minute at 37°C is defined as one unit of enzyme activity.

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

#### 4. Calculated by liquid volume

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

$$\text{FRK activity (U/mL)} = \Delta A \div (\epsilon \times d) \times V_{\text{total reaction}} \times 10^9 \div V_{\text{sample}} \div T = 321.54 \times \Delta A$$

$\epsilon$ : NADH molar extinction coefficient,  $6.22 \times 10^3$  L/mol /cm;

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

$V_{\text{total reaction}}$ : total volume of reaction,  $1 \times 10^{-3}$ L;

$10^9$ : Unit conversion,  $1\text{mol} = 10^9\text{nmol}$ ;

$V_{\text{sample}}$ : Add sample volume, 0.1mL;

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

Cpr: Sample protein concentration, mg/mL;

W: Sample quality, g;

N: Number of cells, measured in 10<sup>6</sup>;

T: Reaction time, 5min.

**Note:**

1. If  $\Delta A$  is less than 0.005, the sample size can be increased or the reaction time can be extended before determination; If the  $\Delta A$  determination is greater than 0.6 or the  $A_{1 \text{ text}}$  determination is less than  $A_{1 \text{ blank}}$ , 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 1mL quartz colorimetric dish to measure and calculate:  $\Delta A_{\text{text}} = A_{2 \text{ text}} - A_{1 \text{ text}} = 0.165 - 0.092 = 0.073$ ,  $\Delta A_{\text{blank}} = A_{2 \text{ blank}} - A_{1 \text{ blank}} = 0.013 - 0.012 = 0.001$ ,  $\Delta A = \Delta A_{\text{text}} - \Delta A_{\text{blank}} = 0.072$ . Calculated by sample quality:  
FRK activity (U/g mass) =  $321.54 \times \Delta A \div W = 226.304$  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 1mL quartz colorimetric dish to measure and calculate:  $\Delta A_{\text{text}} = A_{2 \text{ text}} - A_{1 \text{ text}} = 0.737 - 0.237 = 0.5$ ,  $\Delta A_{\text{blank}} = A_{2 \text{ blank}} - A_{1 \text{ blank}} = 0.013 - 0.012 = 0.001$ ,  $\Delta A = \Delta A_{\text{text}} - \Delta A_{\text{blank}} = 0.499$ . Calculated by sample quality:  
FRK activity (U/g mass) =  $321.54 \times \Delta A \div W = 2674.141$  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 Immunoreactivity 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.

**Related products:**

- BC0740/BC0745 己糖激酶 (HK) 活性检测试剂盒  
BC0530/BC0535 磷酸果糖激酶 (PFK) 活性检测试剂盒  
BC2190/BC2195 磷酸烯醇式丙酮酸羧化酶 (PEPC) 活性检测试剂盒  
BC2450/BC2455 植物组织果糖 (FT) 含量检测试剂盒