（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K1157-M

Specification：48T(46 samples)/96T(94samples)

Measuring instrument：Microplate reader(340 nm)

Detection range：1.61-83.76 U/L

Elabscience® Fructokinase (FRK) Activity Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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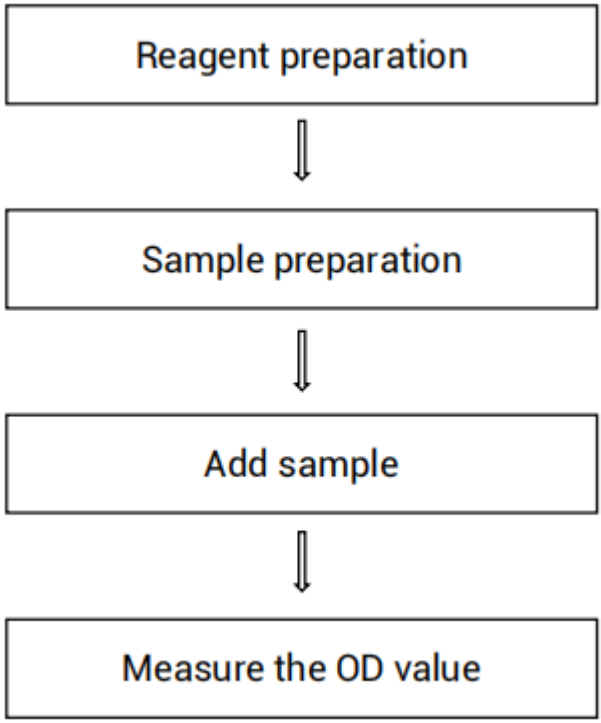
Assay summary



Intended use

This kit can be used to measure fructokinase (FRK) activity in animal tissue, plant tissue and cell samples.

Detection principle

Fructokinase (FRK) is one of the key enzymes in the body's sugar metabolism, catalyzing the conversion of fructose into fructose-6-phosphate (F6P), which then proceeds to the glycolysis pathway and the oxidative pentose pathway.
The detection principle of this kit: The product generated by FRK catalyzing the substrate has the maximum absorption at 340 nm. The FRK enzyme activity is calculated by measuring the OD value at 340 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months
Reagent 2	Buffer Solution	11 mL × 1 vial	22 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Oxidant Reagent	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months shading light
Reagent 4	Enzyme Reagent	0.5 mL × 1 vial	1.0 mL × 1 vial	-20°C, 12 months shading light
Reagent 5	Substrate	0.4 mL × 1 vial	0.8 mL × 1 vial	-20°C, 12 months shading light
	UV-Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (340 nm), Incubator

Regents:

Double distilled water

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of oxidant working solution:

Dissolve one vial of oxidant reagent with 0.75 mL of double distilled water, mix well to dissolve. Store at -20°C for 7 days protected from light.

③ The preparation of measuring working solution:

Before testing, please prepare sufficient mersuring working solution according to the test wells. For example, prepare 1800 μL of mersuring working solution (mix well 1550 μL of buffer solution, 90 μL of oxidant working solution, 90 μL of enzyme reagent and 70 μL of substrate). Keep measuring working solution on ice for use. The solution should be prepared on spot and used up within 8 h.

Sample preparation

① Sample preparation

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M is recommended for animal tissue samples. E-BC-K168-M is recommended for plant tissue samples).

Cell samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Homogenize 1×10^6 cells in 200 μL extraction solution with a ultrasonic cell disruptor at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Yeast powder	10-15
10% Mouse kidney tissue homogenate	5-10
1×10^6 A549 cells	1
1×10^6 HL-60 cells	1
1×10^6 293T cells	1
1×10^6 K562 cells	1
10% Potato tissue homogenate	1
10% Tomato pulp tissue homogenate	1
10% Yellow peach pulp tissue homogenate	1
10% Corn tissue homogenate	1

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Blank well: add 20 μL of extraction solution into blank wells.
Sample well: add 20 μL of sample into sample wells.

② Add 180 μL of measuring working solution into each well.

③ Mix fully for 5 s with microplate reader and measure the OD value of each well at 340 nm as A_1. Incubate at 37°C for 10 min, measure the OD value of each well at 340 nm as A_2, $\Delta A = A_2 - A_1$.

Calculation

The sample:

1. Tissue or cell samples:

Definition: The amount of 1 g tissue or cell protein per 1 min that produce 1 μmol of product at 37 °C is defined as 1 unit.

$$\text{FRK activity (U/gprot)} = \frac{\Delta A_{340} \times V_{total} \times f}{\varepsilon \times d \times V_{sample} \times T \times C_{pr}}$$

[Note]:

ΔA: $A_2 - A_1$.

ΔA_{340}: $\Delta A_{sample} - \Delta A_{blank}$.

ε: The molar extinction coefficient at 340 nm, 6.22×10^-3 L/μmol/cm.

d: Optical path, 0.60 cm.

V_{total}: The volume of reaction system, 0.2 mL.

V_{sample}: The volume of sample, 0.02 mL.

C_{pr}: Concentration of protein in sample, gprot/L.

T: Reaction time, 10 min.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three 10% yeast powder samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	20.00	40.00	80.00
%CV	1.5	1.9	3.3

Inter-assay Precision

Three 10% yeast powder samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	20.00	40.00	80.00
%CV	8.0	8.6	9.6

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 101.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	20.00	40.00	80.00
Observed Conc. (U/L)	20.0	39.6	84.0
Recovery rate (%)	100.0	99.0	105.0

Sensitivity

The analytical sensitivity of the assay is 1.61 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix Π Example Analysis

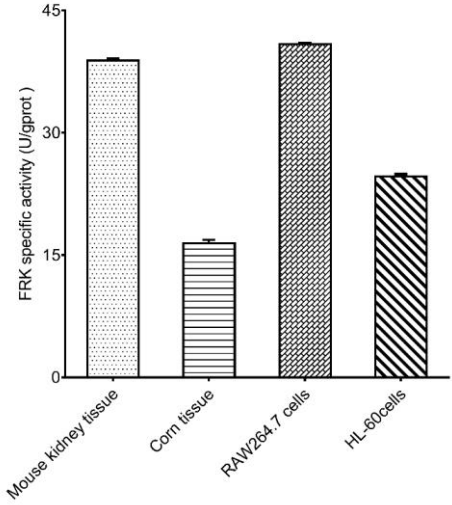
Example analysis：

Take 20 μL of 10% mouse kidney tissue homogenate which dilute for 5 times and carry the assay according to the operation steps. The results are as follows:

The A_1 of the sample well is 0.150, the A_2 of the sample well is 0.534, ΔA_{sample} = 0.534 – 0.150 = 0.384. The A_1 of the blank well is 0.077, the A_2 of the blank well is 0.080, ΔA_{blank} = 0.080 – 0.077 = 0.003, ΔA_{340}= 0.384 – 0.003 = 0.381, the concentration of protein in sample is 13.2 gprot/L, and the calculation result is:

FRK activity (U/gprot) = (0.381×0.2×5) / (6.22×10^-3×0.6×0.02×10×13.2) =38.7U/gprot

Detect 10% mouse kidney tissue homogenate (the concentration of protein is 13.2 gprot/L, dilute for 5 times), 10% corn tissue homogenate (the concentration of protein is 5.36 gprot/L), 1×10^6 RAW264.7 cells (the concentration of protein is 1.62 gprot/L), 1×10^6 HL-60 cells (the concentration of protein is 2.14 gprot/L), according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.