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

Catalog No：E-BC-K857-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（440-460 nm）

Detection range：0.5-70.0 U/L

Elabscience® Uridine Diphosphate Glucose Pyrophosphorylase (UGP) Activity 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
Tell: 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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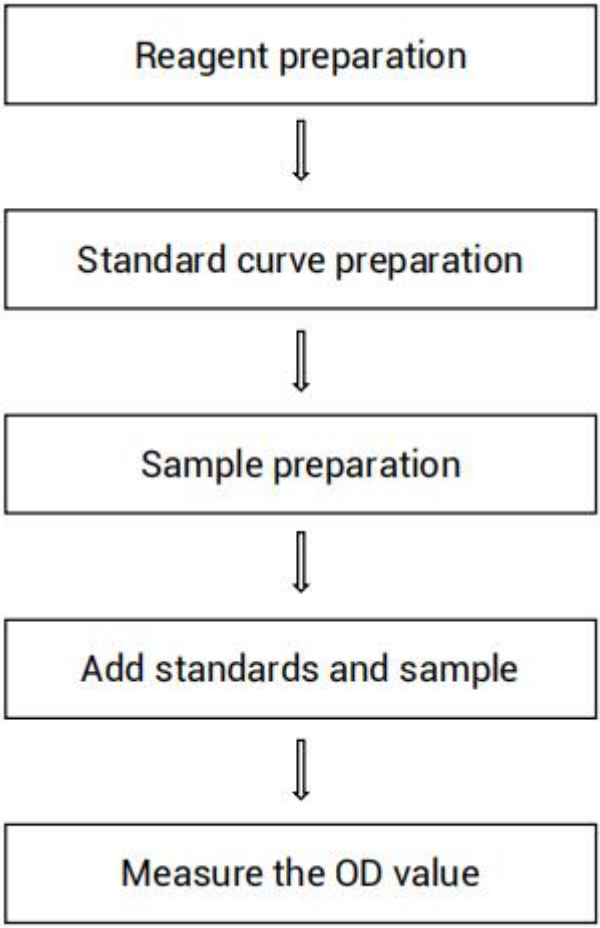
Assay summary



Intended use

The kit can be used to detect the activity of uridine diphosphate glucose prophosphorylase (UGP) in animal and plant tissue samples.

Detection principle

Uridine diphosphate glucose pyrophosphorylase (UGP) catalyzes glucose activation before glycogen synthesis, synthesizing 1-phosphate glucose and UTP molecules into UDP-glucose (UDPG). UDPG is the main form of activating enzyme in higher plants and animals, and participates in the anabolic metabolism of glycogen, sucrose and cellulose as a glucosyl donor.

Uridine diphosphate glucose pyrophosphorylase (UGP) catalyzes the synthesis of 1-phosphoglucose in the sample into UDP-glucose, and UDP-glucose is catalyzed by the enzyme to produce a color producing substance, which has a maximum absorption peak at about 450 nm. The enzyme activity can be calculated by the increase of OD value at 450 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	50 mL×1 vial	-20℃, 12 months
Reagent 2	Buffer Solution	20 mL×1 vial	-20℃, 12 months
Reagent 3	Enzymatic Reagent	1.2 mL×2 vials	-20℃, 12 months, shading light
Reagent 4	Substrate A	Powder×1 vial	-20℃, 12 months, shading light
Reagent 5	Substrate B	1.5 mL×1 vial	-20℃, 12 months, shading light
Reagent 6	Accelerant	1.5 mL×1 vial	-20℃, 12 months, shading light
Reagent 7	Chromogenic Agent	2.5 mL×1 vial	-20℃, 12 months, shading light
Reagent 8	0.5 mmol/L Standard Solution	3.3 mL×1 vial	-20℃, 12 months, shading light
	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 (440-460 nm, optimum wavelength: 450 nm), Vortex mixer

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate A working solution:
Dissolve one vial of substrate A with 0.8 mL of double distilled water, mix well to dissolve. Store at -20°C for 4 weeks.

③ The preparation of measuring working solution:
For each well, prepare 120 μL of measuring working solution (mix well 72 μL of buffer solution, 18 μL of enzymatic reagent, 6 μL of substrate A working solution, 12 μL of substrate B and 12 μL of accelerant).
Store at 2-8°C for 1 week protected from light.

④ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 0.5 mmol/L standard solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.15, 0.2, 0.3, 0.35, 0.4, 0.5 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.10**	**0.15**	**0.20**	**0.30**	**0.35**	**0.4**	**0.50**
0.5 mmol/L standard (μL)	0	40	60	80	120	140	160	200
Double distilled water (μL)	200	160	140	120	80	60	40	0

Sample preparation

① Sample preparation:

Tissue samples:

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

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ E-BC-K318-M is recommended for animal tissue samples.
E-BC-K168-M is recommended for plant tissue samples.

② Dilution of sample

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

Sample type	Dilution factor
10% Corn tissue homogenate	180-200
10% Patato tissue homogenate	30-40
10% Sweet patato tissue homogenate	100-120
10% Corn husk tissue homogenate	35-45
10% Epipremnum aureum tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat liver tissue homogenate	100-120

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

Operating steps

① Sample well: add 20 μL of sample into sample wells.
Control well: add 20 μL of sample into control wells.
Standard well: add 20 μL of standard with different concentrations into standard wells.

② Add 120 μL of measuring working solution to standard wells and sample wells. Add 120 μL of buffer solution to control wells.

③ Add 20 μL of chromogenic agent to each wells.

④ Mix fully for 5 s with microplate reader and incubate at 37℃ for 5 min with shading light. Measure the OD value of each well at 450 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Tissue sample

Definition: The amount of uridine diphosphate glucose prophosphorylase(UGP) in 1 g sample protein that hydrolyze the substrate to produce 1 μmol product in 1 minute at 37°C is defined as 1 unit.

$$\text{UGP activity (U/gprot)} = (\Delta A_{450} - b) \div a \div T \times f \times 1000 \div C_{pr}$$

[Note]

ΔA_{450}: $\Delta A_{450} = OD_{sample} - OD_{control}$.

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

1000: 1 mmol/L = 1000 μmol/L.

f: Dilution factor of the sample before tested.

T: The incubation time, 5 min.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat liver tissue 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)	2.60	16.50	35.60
%CV	3.2	3.0	1.0

Inter-assay Precision

Three rat liver tissue 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)	2.60	16.50	35.60
%CV	2.3	3.9	2.8

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 100.5%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.13	0.25	0.38
Observed Conc. (mmol/L)	0.1	0.3	0.4
recovery rate(%)	100	101	100.5

Sensitivity

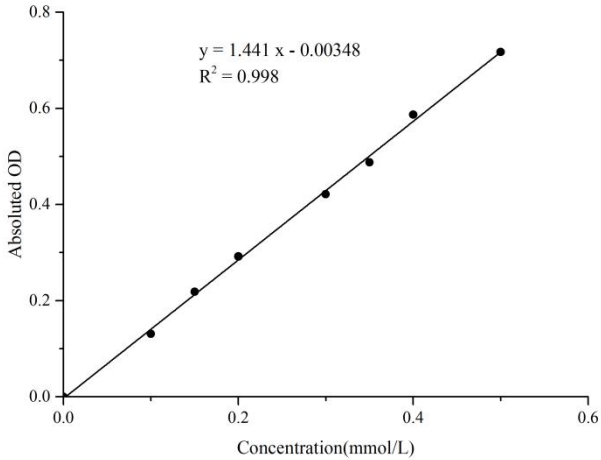
The analytical sensitivity of the assay is 0.5 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding

concentration.

2. Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only.

Concentration (mmol/L)	**0**	**0.1**	**0.15**	**0.2**	**0.3**	**0.35**	**0.4**	**0.5**
OD value	0.063	0.195	0.283	0.351	0.485	0.552	0.648	0.78
	0.063	0.193	0.28	0.359	0.484	0.549	0.652	0.78
Average OD	0.063	0.194	0.281	0.355	0.484	0.550	0.65	0.78
Absoluted OD	0	0.131	0.218	0.292	0.421	0.487	0.587	0.717



Appendix Π Example Analysis

Example analysis：

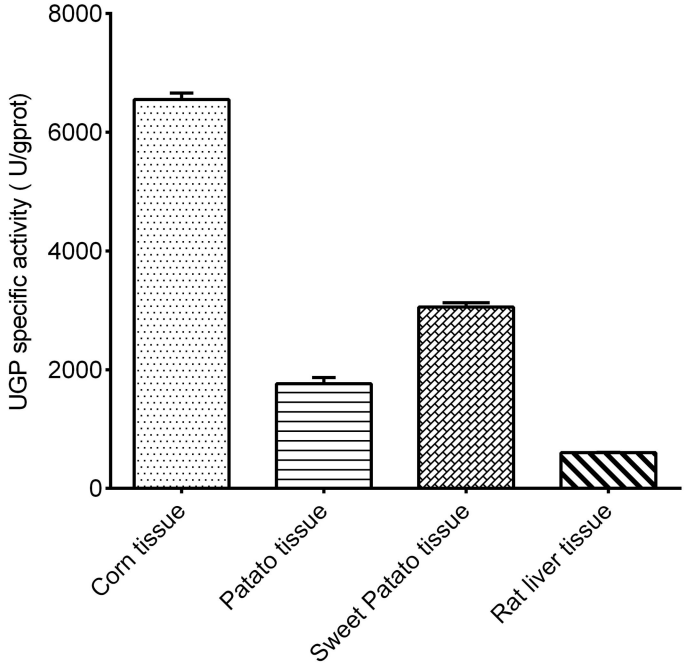
Dilute 10% patato tissue homogenate, dilute for 40 times, then take 20 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 1.441 x - 0.00348. The OD value of control well is 0.055. The OD value of sample well is 0.537, ΔA_{450} = 0.537 - 0.055 = 0.482, the concentration of protein in sample is 1.53 gprot/L, and the calculation result is:

UGP activity (U/gprot) = (0.482 + 0.00348) ÷ 1.441 ÷ 5 × 1000 × 40 ÷ 1.53

= 1761.59 U/gprot

Detect 10% corn tissue homogenate (the concentration of protein is 1.60 gprot/L, dilute for 200 times), 10% patato tissue homogenate (the concentration of protein is 1.53 gprot/L, dilute for 40 times), 10% sweet patato tissue homogenate (the concentration of protein is 2.07 gprot/L, dilute for 100 times) and 10% rat liver tissue homogenate (the concentration of protein is 11.05 gprot/L, dilute for 100 times)according to the protocol, the result is as follows：


8000
6000
4000
2000
0
UGP specific activity (U/gprot)
Corn tissue
Patato tissue
Sweet Patato tissue
Rat liver tissue

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.