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

Catalog No：E-BC-K856-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（440-460 nm）

Detection range：0.5-81.59 U/L

Elabscience®Adenosine Diphosphate Glucose Prophosphorylase(AGP) 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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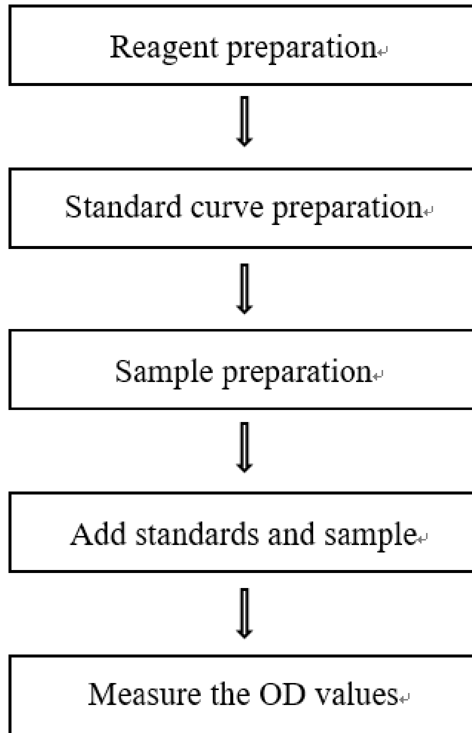
Assay summary



Intended use

This kit can measure adenosine diphosphate glucose prophosphorylase (AGP) activity in plant tissue samples.

Detection principle

Adenosine diphosphate glucose pyrophosphorylase (AGP) catalyzes the substrate to produce an intermediate product, which produce chromogenic substances after enzyme catalysis. The substance has a maximum absorption peak at around 450 nm, and the AGP activity can be calculated by the OD value at 450 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20°C, 12 months
Reagent 2	Enzymatic Reagent	1.56 mL × 1 vial	-20°C, 12 months
Reagent 3	Substrate A	Powder × 1 vial	-20°C, 12 months
Reagent 4	Substrate B	2.5 mL × 1 vial	-20°C, 12 months
Reagent 5	Accelerant A	2.5 mL × 1 vial	-20°C, 12 months
Reagent 6	Chromogenic Agent	2.5 mL × 1 vial	-20°C, 12 months, shading light
Reagent 7	Accelerant B	2.5 mL × 1 vial	-20°C, 12 months
Reagent 8	0.5 mmol/L Standard Solution	3.3 mL × 1 vial	-20°C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

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, Incubator

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of substrate A working solution:

Dissolve one vial of substrate A with 0.6 mL of double distilled water, mix well to dissolve. Store at -20 ℃ for 1 month.

③ Preparation of measuring working solution:

Before testing, please prepare sufficient mersuring working solution according to the test wells. For example, prepare 177 μL of mersuring working solution (mix well 120 μL of buffer solution, 12 μL of enzymatic reagent, 5 μL of substrate A working solution, 20 μL of accelerant A and 20 μL of accelerant B). Store at -20 ℃ for 1 week protected from light.

④ Preparation of chromogenic working solution:

For each well, prepare 40 μL of chromogenic working solution (mix well 20 μL of substrate B and 20 μL of chromogenic agent). The chromogenic working solution should be prepared on spot.

⑤ Preparation of control working solution:

Before testing, please prepare sufficient control working solution according to the test wells. For example, prepare 180 μL of control working solution (mix well 140 μL of buffer solution, 20 μL of accelerant A and 20 μL of accelerant B). Store at -2-8 ℃ for 1 week protected from light.

⑥ 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 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**	**0.1**	**0.15**	**0.2**	**0.3**	**0.35**	**0.4**	**0.5**
0.5 mmol/L standard (μL)	0	20	60	80	120	140	160	200
Double distilled water (μL)	200	180	140	120	80	60	40	0

Sample preparation

① **Sample preparation**

Tissue sample:

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 normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

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

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M).

➁ **Dilution of sample**

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

Sample type	Dilution factor
10% Corn tissue homogenate	1
10% Epipremnum aureum tissue homogenate	1
10% Asparagus tissue homogenate	1
10% Barley tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Add 20 μL of standard solution with different concentrations into the corresponding wells.

Sample well: Add 20 μL of sample into the corresponding wells.

Control well: Add 20 μL of sample into the corresponding wells.

② Add 140 μL of measuring working solution into standard well and sample well. Add 160 μL of control working solution into control well.

③ Add 40 μL of chromogenic working solution into standard well and sample well.

④ Add 20 μL of chromogenic agent into control well.

⑤ Mix fully with microplate reader for 5 s, incubate at 37°C with shading light for 5 min. Measure the OD values 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 AGP in 1 g tissue protein per minute that catalyze the substrate to produce 1 μmol substance at 37°C is defined as 1 unit

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

[Note]

ΔA_{450}: $OD_{Sample} - OD_{Control}$.

T: The time of reaction, 5 min.

Cpr: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

1000： 1 mmol/L=1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three barley 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)	4.80	26.70	66.50
%CV	4.2	3.3	3.0

Inter-assay Precision

Three barley 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)	4.80	26.70	66.50
%CV	5.5	10.0	7.6

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 99%.

	Standard 1	Standard 2	Standard 3
Expected Conc. （mmol/L）	0.13	0.25	0.38
Observed Conc. （mmol/L）	0.1	0.2	0.4
recovery rate(%)	102	98	97

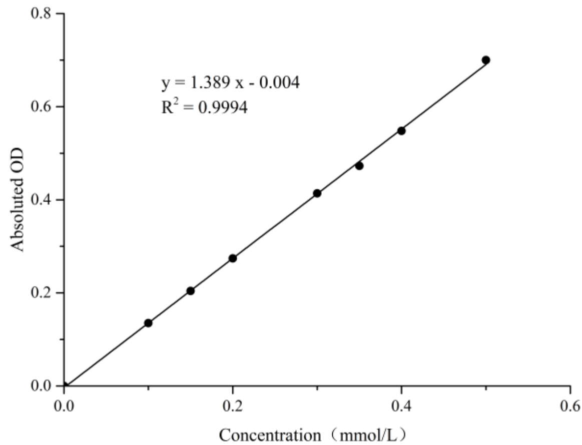
Sensitivity

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	0.083	0.212	0.284	0.353	0.494	0.562	0.629	0.783
	0.078	0.218	0.285	0.355	0.494	0.545	0.627	0.778
Average OD	0.081	0.215	0.285	0.354	0.494	0.554	0.628	0.781
Absoluted OD	0.000	0.135	0.204	0.274	0.414	0.473	0.548	0.700



Appendix Ⅱ Example Analysis

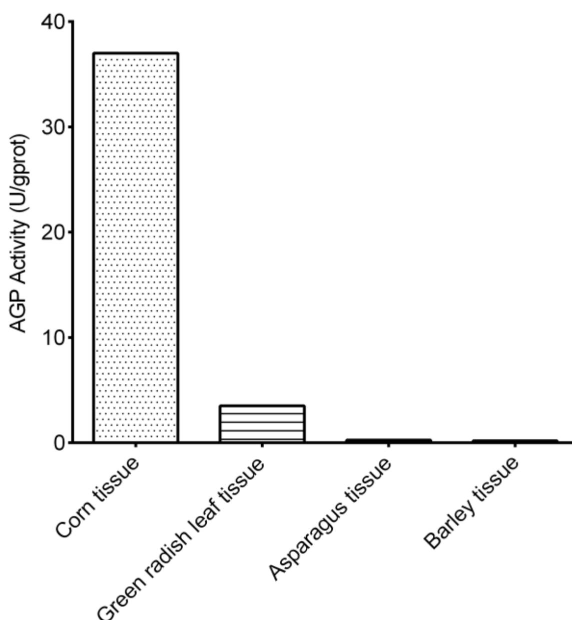
Example analysis:

Take 20 μL of 10% corn tissue homogenate and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 1.389 x – 0.004, the average OD value of the control is 0.086, the average OD value of the sample is 0.218, ΔA450 = 0.218 – 0.086 = 0.132, the concentration of protein in sample is 0.52 gprot/L, and the calculation result is:

$$\text{AGP activity (U/gprot)} = (0.132 + 0.004) \div 1.389 \div 5 \div 0.52 \times 1000 = 37.66 \text{ U/gprot}$$

Detect 10% corn tissue homogenate (the concentration of protein is 0.52 gprot/L), 10% green radish leaf tissue homogenate (the concentration of protein is 0.14 gprot/L), 10% asparagus tissue homogenate (the concentration of protein is 0.77 gprot/L), 10% barley tissue homogenate (the concentration of protein is 1.24 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.