ADPG Pyrophosphorylase (AGP) Activity Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer/Microplate Reader

Catalog Number: BC0435

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 100 mL×1	2-8℃
Reagent I	Liquid 20 mL×1	2-8℃
Reagent II A	Powder×2	-20℃
Reagent II B	Liquid 12mL×1	2-8℃
Reagent III	Powder×2	2-8℃
Reagent IV	Powder×2	-20℃
Reagent V	Powder×1	-20℃

Solution Preparation:

1. **Reagent II B**: Before use, add 5mL Reagent II B to a bottle of Reagent II A to fully dissolve. The unused reagents are subpackaged and stored at -20℃ for 4 weeks. Avoid repeated freezing and thawing during storage.
2. **Reagent III:** Dissolve one Reagent III with 3 mL distilled water. The unused reagents are subpackaged and stored at -20℃ for 4 weeks. Avoid repeated freezing and thawing during storage.
3. **Reagent IV**: Dissolve one Reagent IV with 500μL distilled water. The unused reagents are subpackaged and stored at -20℃ for 4 weeks. Avoid repeated freezing and thawing during storage (This reagent is a freeze-dried reagent, and there may be significant or even small differences in the amount of reagents observed by the naked eye between different bottles. This phenomenon does not affect the use, and the actual quality is the same).
4. **Reagent V:** Add 500μL of distilled water to fully dissolve it. The unused reagents are subpackaged and stored at -20℃ for 4 weeks. Avoid repeated freezing and thawing during storage (This reagent is a freeze-dried reagent, and there may be significant or even small differences in the amount of reagents observed by the naked eye between different bottles. This phenomenon does not affect the use, and the actual quality is the same).

Product Description:

ADPG Pyrophosphorylase (AGP) (EC 2.7.7.21) exists mainly in plants, is the main rate-limiting step in plant starch biosynthesis, which catalyzes the reaction of glucose-1-phosphate

(G1P) with ATP to

produce direct precursor adenosine diphosphate glucose (ADPG) for starch synthesis.

AGP catalyzes the reverse reaction to produce G1P, the added phosphate hexose mutase and 6-phosphate glucose dehydrogenase catalyze the formation of 6-phosphate gluconate and NADPH. In this kit, the activity of AGP is determined by the increase rate of NADPH at 340 nm.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, water bath/incubator, centrifuge, adjustable pipette, micro quartz cuvette/96 well UV plate, mortar/homogenizer, ice, and distilled water.

Procedure:

I. Sample preparation:

Add 1 mL of Extract solution to 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 10000 ×g for 10 minutes at 4℃, and take the supernatant on ice before test.

II. Determination procedure:

1. Preheat microplate reader or spectrophotometer for 30 minutes, adjust the wavelength to 340 nm, spectrophotometer set zero with distilled water.
2. Add the following reagents.

Reagent (μL)	Test tube (T)
Reagent I	40
Reagent II	64
Sample	8
Mix thoroughly and incubate at 30℃ for 15 minutes, then place the tubes in a boiling water bath for 1 minute (cover tightly to prevent moisture loss). After the ice bath cools rapidly, add the following reagents. (keep the temperature of Reagent I and III at 37℃ for more than 10 min.)	
Reagent I	120
Reagent III	40
Reagent IV	8
Reagent V	4

Mix thoroughly and timing, Determination of 10s absorbance A1 and 130s absorbance A2 at 340nm wavelength, record as A1 (10s) and A2 (130s) respectively. ΔA=A2-A1.

III. Calculation:

A. micro quartz cuvette

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per minute every milligram of protein.

AGP (U/mg prot)=[ΔA÷(ε×d)×Vrv]÷(Vs×Cpr)÷T=380.5×ΔA÷Cpr

Note: This method requires the determination of the protein concentration of the crude enzyme solution.

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per minute every gram of tissue.

AGP (U/g weight)=[ΔA÷(ε×d)×Vrv]÷(W÷Ve×Vs)÷T=380.5×ΔA÷W

T: Reaction time, 15 minutes;

ε: NADPH extinction coefficient, 6.22×10^{-3} mL/nmol/cm;

Vrv: Total reaction volume,0.284 mL;

d: Light path of cuvette, 1 cm;

Cpr: Sample protein concentration (mg/mL);

Vs: Supernatant volume, 0.008 mL;

Ve: Extract volume, 1 mL;

W: Sample weight (g).

B. 96 well UV plate

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per minute every milligram of protein.

AGP (U/mg prot)=[ΔA÷(ε×d)×Vrv]÷(Vs×Cpr)÷T=634.1×ΔA÷Cpr

Note: This method requires the determination of the protein concentration of the crude enzyme solution.

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per minute every gram of tissue.

AGP (U/g weight)=[ΔA÷(ε×d)×Vrv]÷(W÷Ve×Vs)÷T=634.1×ΔA÷W

T: Reaction time, 15 minutes;

ε: NADPH molar extinction coefficient, 6.22×10^{-3} mL/nmol/cm;

Vrv: Total reaction volume,0.284 mL;

d: Light path of cuvette, 0.6 cm;

Cpr: Sample protein concentration (mg/mL);

Vs: Supernatant volume, 0.008 mL;

Ve: Extract solution, 1 mL;

W: Sample weight (g).

Note:

1. If there are many samples for one-time determination, Reagent I and Reagent II can be proportioned into mixture 1, and Reagent I, Reagent III, Reagent IV and Reagent V can be proportioned into mixture 2.

2. If the measured value is small, the sample size can be increased or the reaction time of the second step
   can be extended.

Experimental example:

1. Take 0.1g of willow and add 1 mL of Extract solution to homogenize in ice bath. After centrifugation at 10000 ×g for 10 minutes at 4℃, the supernatant is put on ice, and then the determination procedure is followed by micro quartz colorimetric plate. ΔA = A2-A1 = 0.5784-0.4855 = 0.0929
   AGP activity (U/g mass) = 380.5×ΔA ÷ W = 353.48 U/g mass.

Recent Product Citations:

[1] Zhu L, Li Y, Wang C, Wang Z, Cao W, Su J, Peng Y, Li B, Ma B, Ma F, Ruan YL, Li M. The SnRK2.3-AREB1-TST1/2 cascade activated by cytosolic glucose regulates sugar accumulation across tonoplasts in apple and tomato. Nat Plants. 2023 Jun;9(6):951-964. doi: 10.1038/s41477-023-01443-8. Epub 2023 Jun 8. PMID: 37291399.

[2] Shi W, Ma Q, Yin W, Liu T, Song Y, Chen Y, Song L, Sun H, Hu S, Liu T, Jiang R, Lv D, Song B, Wang J, Liu X. The transcription factor StTINY3 enhances cold-induced sweetening resistance by coordinating starch resynthesis and sucrose hydrolysis in potato. J Exp Bot. 2022 Aug 11;73(14):4968-4980. doi: 10.1093/jxb/erac171. PMID: 35511088.

[3] Xiao X, Wang Q, Ma X, Lang D, Guo Z, Zhang X. Physiological Biochemistry-Combined Transcriptomic Analysis Reveals Mechanism of Bacillus cereus G2 Improved Salt-Stress Tolerance of Glycyrrhiza uralensis Fisch. Seedlings by Balancing Carbohydrate Metabolism. Front Plant Sci. 2022 Jan 4; 12:712363. doi: 10.3389/fpls.2021. 712363. PMID: 35058941; PMCID: PMC8764457.

References:

[1] Baroja-Fernández E, Zandueta-Criado A, Rodríguez-López M, et al. Distinct isoforms of ADPglucose pyrophosphatase and ADPglucose pyrophosphorylase occur in the suspension-cultured cells of sycamore (Acer pseudoplatanus L) [J]. FEBS letters, 2000, 480(2-3): 277-282.

[2] McCoy JG, Arabshahi A, Bitto E, et al. Structure and mechanism of an ADP-glucose phosphorylase from Arabidopsis thaliana [J]. Biochemistry, 2006, 45(10): 3154-3162.

[3] Li X, Shen CR, Liao JC. Isobutanol production as an alternative metabolic sink to rescue the growth deficiency of the glycogen mutant of Synechococcus elongatus PCC 7942 [J]. Photosynthesis Research, 2014, 120(3): 301-310.

Related products:

BC0700/BC0705	Starch Content Assay Kit
BC1850/BC1855	Soluble Starch Synthase(SSS) Activity Assay Kit
BC3290/BC3295	Bound Station amylosynthease Activity Assay Kit
BC2670/BC2675	α-1,4-Glucan Glucohydrolace Activity Assay Kit

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