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

Catalog Number: BC0430

Size:50T/48S

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 50 mL×1	2-8℃
Reagent I	Liquid 20 mL×1	2-8℃
Reagent II A	Powder×2	-20℃
Reagent II B	Liquid 12 mL×1	2-8℃
Reagent III	Powder×2	2-8℃
Reagent IV	Powder×2	-20℃
Reagent V	Powder×2	-20℃

Solution Preparation:

1. **Reagent II:** 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 it with 500 μL of distilled water before use. 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:** Dissolve it with 500μL distilled water before use. 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, water bath/constant temperature incubator, centrifuge, adjustable pipette, 1mL quartz cuvette, 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℃, take the supernatant and place it on ice for testing.

II. Determination procedure:

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

Reagent (μL)	Test tube (T)
Reagent I	100
Reagent II	160
Sample	20
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) and rapid cooling by ice bath. (keep the temperature of Reagent I and III at 37℃ for more than 10 min.)	
Reagent I	300
Reagent III	100
Reagent IV	20
Reagent V	10

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:

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)=[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,0.71 mL;

d: Light path of cuvette, 1 cm;

Cpr: Sample protein concentration (mg/mL);

Vs: Supernatant volume, 0.02 mL;

Ve: Extract solution volume, 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 leaves 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 1 mL quartz cuvette. ΔA = A2-A1 = 0.55-0.491=0.059. AGP activity (U/g mass) = 380.5×ΔA ÷ W = 224.495 U/g mass.

Recent Product Citations:

[1] Yang Q, Ding J, Feng X, Zhong X, Lan J, Tang H, Harwood W, Li Z, Guzmán C, Xu Q, Zhang Y, Jiang Y, Qi P, Deng M, Ma J, Wang J, Chen G, Lan X, Wei Y, Zheng Y, Jiang Q. Editing of the starch synthase IIa gene led to transcriptomic and metabolomic changes and high amylose starch in barley. Carbohydr Polym. 2022 Jun 1; 285:119238. doi: 10.1016/j.carbpol.2022.119238. Epub 2022 Feb 10. PMID: 35287861.

[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 ADP-glucose pyrophosphatase and ADP-glucose 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:

BC1850/BC1855	Soluble Starch Synthase(SSS) Activity Assay Kit
BC3290/BC3295	Bound Station Amylosynthease Activity Assay Kit

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