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ADPG Pyrophosphorylase(AGP) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: AK0320

Size: 100T/96S

Components:

Extract solution: 100 mL×1. Store at 4℃ .

Reagent I: 20 mL×1. Store at 4℃ .

Reagent II: Powder×1. Store at -20℃ . Dissolve with 6.4 mL of distilled water before use. Unused reagent is still stored at -20℃ .

Reagent III: Powder×2. Store at 4℃ . Dissolve with 2 mL of distilled water before use. Unused reagent is stored at -20℃ .

Reagent IV: Powder×2. Store at -20℃ . Dissolve with 500 μL of distilled water before use. Unused reagent is still stored at -20℃ .

Reagent V: 250 μL×2. Store at -20℃ .

Product Description:

ADPG Pyrophosphorylase(AGP) 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:

Spectrophotometer/Microplate Reader, desk centrifuge, adjustable pipette, water bath, micro quartz cuvette/96 well flat-bottom UV plate, mortar/homogenizer, ice, 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℃ to remove insoluble materials, 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, 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) and rapid cooling by ice bath. (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

Detect the absorbance at 340 nm detect the absorbance of initial and final reaction (2 min) at 340 nm, record as A1 (0 s) and A2 (2 min) 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

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

d: Light path of cuvette, 1 cm;

Vrv: Total reaction volume,0.284 mL;

Vs: Supernatant volume, 0.008 mL;

Ve: Extract volume, 1 mL;

Cpr: Sample protein concentration (mg/mL);

T: Reaction time, 15 minutes;

W: Sample weight(g).

B. 96 well flat-bottom 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

ε: NADH molar extinction coefficient, 6.22×10^{-3} mL/nmol/cm;
d: Light path of cuvette, 0.6 cm;
Vrv: Total reaction volume,0.284 mL;
Vs: Supernatant volume, 0.008 mL;
Ve: Extract volume, 1 mL;
Cpr: Sample protein concentration (mg/mL);
T: Reaction time, 15 minutes;
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.

Experimental example:

1. Take 0. 1g of willow and add 1 mL of Extract solution to homogenize in ice bath. After centrifugation at 4℃ for 10 min, 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.

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.

Related products:

AK0520/AK0519	Starch Content Assay Kit
AK0413/AK0615	Soluble Starch Synthase(SSS) Activity Assay Kit
AK0364/AK0363	Bound Station amylosynthease Activity Assay Kit
AK0187/AK0186	α- 1,4-Glucan Glucohydrolace Activity Assay Kit