

ADPG Pyrophosphorylase(AGP) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC0430

Size: 50T/48S

Components:

Extract solution: 50 mL×1. Store at 4°C.

Reagent I: 20 mL×1. Store at 4°C.

Reagent II: Powder×1. Store at -20°C. Dissolve with 8 mL of distilled water before use. Unused reagent is still stored at -20°C.

Reagent III: Powder×2. Store at 4°C. Dissolve with 3 mL of distilled water before use. Unused reagent is stored at -20°C.

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

Reagent V: 250 μL×2. Store at -20°C.

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, desk centrifuge, adjustable pipette, water bath, 1 mL quartz cuvette, 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°C to remove insoluble materials, and take the supernatant on ice before test.

II. Determination procedure:

1. Preheat 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)
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Reagent I	100
Reagent II	160
Sample	20
Mix thoroughly and incubate at 30°C 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°C for more than 10 min.)	
Reagent I	300
Reagent III	100
Reagent IV	20
Reagent V	10

Mix thoroughly and timing, detect the absorbance at 340 nm detect the absorbance of initial and final reaction at 340 nm, record as A1(0s) and A2(2min) respectively. $\Delta A = A_2 - A_1$.

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.

$$AGP \text{ (U/mg prot)} = [\Delta A \div (\epsilon \times d) \times V_{rv}] \div (V_s \times C_{pr}) \div T = 380.5 \times \Delta A \div C_{pr}$$

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.

$$AGP \text{ (U/g)} = [\Delta A \div (\epsilon \times d) \times V_{rv}] \div (W \div V_e \times V_s) \div T = 380.5 \times \Delta A \div W$$

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

d: Light path of cuvette, 1 cm;

V_{rv} : Total reaction volume, 0.71 mL;

V_s : Supernatant volume, 0.02 mL;

V_e : Extract solution volume, 1 mL;

C_{pr} : 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°C for 10 min, the supernatant is put on ice, and then the determination procedure is followed by micro quartz colorimetric plate. $\Delta A = A_2 - A_1 = 0.55 - 0.491 = 0.059$

AGP activity (U/g mass) = $380.5 \times \Delta A \div W = 224.495$ U/g mass.

References:

[1] Baroja-Fernández E, Zandúeta-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:

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