

ADPG Pyrophosphorylase

Microplate Assay Kit

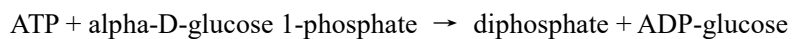
Cat #: orb545616 (manual)

Detection and Quantification of ADPG Pyrophosphorylase Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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INTRODUCTION

In enzymology, a glucose-1-phosphate adenylyltransferase (EC 2.7.7.27) is an enzyme that catalyzes the chemical reaction



Thus, the two substrates of this enzyme are ATP and alpha-D-glucose 1-phosphate, whereas its two products are diphosphate and ADP-glucose.

This enzyme belongs to the family of transferases, specifically those transferring phosphorus-containing nucleotide groups (nucleotidyltransferases).

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Diluent	20 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Substrate	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

Enzyme: add 10 ml diluent to dissolve before use.

Substrate: add 10 ml diluent to dissolve before use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

ASSAY PROCEDURE

Add following reagents into the centrifuge tube:

Reagent	Sample
Sample	50 μ l
Substrate	100 μ l
Mix, incubate at 30°C for 30 minutes, put it into boiling water for 2 minutes. Then keep it on ice for cold. Centrifuged at 10000g 4 °C for 10 minutes, add the supernatant into the microplate.	
Supernatant	100 μ l
Enzyme	100 μ l
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.	

Note:

1) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

CALCULATION

Unit Definition: One Unit of ADPG Pyrophosphorylase activity is defined as the enzyme produces 1 nmol NADPH per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{AGPase (U/mg)} &= (\text{OD}_{\text{Sample (130S)}} - \text{OD}_{\text{Sample (10S)}}) / (\epsilon \times d) \times V_{\text{Total}} \times 10^9 / (V_{\text{Sample}} \times C_{\text{Protein}}) / T1 / T2 \times 1.5 \\ &= 26.8 \times (\text{OD}_{\text{Sample (130S)}} - \text{OD}_{\text{Sample (10S)}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{AGPase (U/g)} &= (\text{OD}_{\text{Sample (130S)}} - \text{OD}_{\text{Sample (10S)}}) / (\epsilon \times d) \times V_{\text{Total}} \times 10^9 / (W \times V_{\text{Sample}} / V_{\text{Assay}}) / T1 / T2 \times 1.5 \\ &= 26.8 \times (\text{OD}_{\text{Sample (130S)}} - \text{OD}_{\text{Sample (10S)}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{AGPase (U}/10^4) &= (\text{OD}_{\text{Sample (130S)}} - \text{OD}_{\text{Sample (10S)}}) / (\epsilon \times d) \times V_{\text{Total}} \times 10^9 / (N \times V_{\text{Sample}} / V_{\text{Assay}}) / T1 / T2 \times \\ & \quad 1.5 \\ &= 26.8 \times (\text{OD}_{\text{Sample (130S)}} - \text{OD}_{\text{Sample (10S)}}) / N \end{aligned}$$

ϵ : molar extinction coefficient, 6.22×10^3 L/mol/cm;

d: the optical path of 96-Well microplate, 0.6 cm;

C_{Protein} : the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

V_{Total} : the total volume of the enzymatic reaction, 0.2 ml;

V_{Sample} : the volume of sample, 0.05 ml;

V_{Assay} : the volume of Assay buffer, 1 ml;

T1: the reaction time, 30 minutes.

T2: the reaction time, 2 minutes.



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