ADPG Pyrophosphorylase

Microplate Assay Kit

User Manual

Catalog # CAK1131

(Version 1.2A)

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

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

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

ATP + alpha-D-glucose 1-phosphate → diphosphate + ADP-glucose

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).

II. 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
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Note:

Enzyme: add 10 ml diluent to dissolve before use.

Substrate: add 10 ml diluent to dissolve before use.

III. 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

IV. 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.

V. ASSAY PROCEDURE

Add following reagents into the centrifuge tube:

Reagent	Sample
Sample	50 µl
Substrate	100 µl
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 µl
Enzyme	100 µl
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.

2) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

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

1. According to the protein concentration of sample

$$AGPase\ (U/mg) = (OD_{Sample(130S)} - OD_{Sample(10S)}) / (\varepsilon \times d) \times V_{Total} \times 10^9 / (V_{Sample} \times C_{Protein}) / T1 / T2 \times 1.5$$

$$= 0.0268 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / C_{Protein}$$

2. According to the weight of sample

$$AGPase\ (U/g) = (OD_{Sample(130S)} - OD_{Sample(10S)}) / (\varepsilon \times d) \times V_{Total} \times 10^9 / (W \times V_{Sample} / V_{Assay}) / T1 / T2 \times 1.5$$

$$= 0.0268 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / W$$

3. According to the quantity of cells or bacteria

$$AGPase\ (U/10^4) = (OD_{Sample(130S)} - OD_{Sample(10S)}) / (\varepsilon \times d) \times V_{Total} \times 10^9 / (N \times V_{Sample} / V_{Assay}) / T1 / T2 \times 1.5$$

$$= 0.0268 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / N$$

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.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES