

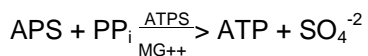


SIGMA QUALITY CONTROL TEST PROCEDURE

Product Information

Enzymatic Assay of ADENOSINE 5'-TRIPHOSPHATE SULFURYLASE (EC 2.7.7.4)

PRINCIPLE:



Abbreviations used:

APS = Adenosine 5'-Phosphosulfate

PP_i = Inorganic Pyrophosphate

ATPS = Adenosine 5'-Triphosphate Sulfurylase

ATP = Adenosine 5'-Triphosphate

HK = Hexokinase

ADP = Adenosine 5'-Diphosphate

β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form

G-6-PDH = Glucose 6-Phosphate Dehydrogenase

6-PG = 6-Phospho-D-Gluconate

β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

CONDITIONS: T = 30°C, pH 8.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 400 mM Tris Buffer, pH 8.0 at 30°C
(Prepare 50 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 30°C with 1 M Acetic Acid.)
- B. 200 mM β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form, Solution (β-NADP)
(Prepare 1 ml in deionized water using β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505.)

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REAGENTS: (continued)

- C. 1 M Magnesium Acetate Solution (MgOAC)
(Prepare 1 ml in deionized water using Magnesium Acetate, Tetrahydrate, Sigma Prod. No. M-9147.)
- D. 1 M D-Glucose Solution (Glucose)
(Prepare 2 ml in deionized water using D-(+)Glucose, Anhydrous, Sigma Prod. No. G-8270.)
- E. 20 mM Adenosine 5'-Phosphosulfate (APS)
(Immediately before use, prepare 1 ml in deionized water using Adenosine 5'-Phosphosulfate, Sodium Salt, Sigma Prod. No. A-5508.)
- F. 100 mM Pyrophosphate Solution, pH 8.0 at 30°C (PP_i)
(Prepare 5 ml in deionized water using Tetrasodium Pyrophosphate, Decahydrate, Sigma Prod. No. P-9146. Adjust to pH 8.0 at 30°C with 1 M HCl.)
- G. Hexokinase and Glucose 6-Phosphate Dehydrogenase Enzyme Solution (HK/G-6-PDH)
(Immediately before use, prepare a solution containing approximately 20 units/ml of Glucose 6-Phosphate Dehydrogenase using Hexokinase and Glucose 6-Phosphate Dehydrogenase, Sigma Prod. No. H-8629 in cold deionized water.)
- H. Adenosine 5'-Triphosphate Sulfurylase Enzyme Solution (ATPS)
(Immediately before use, prepare a solution containing 0.2 - 0.6 unit/ml of Adenosine 5'-Triphosphate Sulfurylase in cold deionized water.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container.

Reagent A (Buffer)	34.00
Reagent B (β-NADP)	0.34
Reagent C (MgOAC)	0.28
Deionized Water	65.38

Mix by swirling. Adjust to pH 8.0 at 30°C if necessary with either 1 M HCl or 1 M NaOH.

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PROCEDURE: (continued)

Pipette (in milliliters) the following reagents into suitable cuvettes:

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.50	2.50
Reagent D (Glucose)	0.10	0.10
Reagent G (HK/G-6-PDH)	0.10	0.10
Reagent E (APS)	0.05	0.05
Reagent H (ATPS)	0.10	-----
Deionized Water	-----	0.10

Mix by inversion and equilibrate to 30°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent F (P _i)	0.10	0.10
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Immediately mix by inversion and record the increase in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{340\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(2.95)(\text{df})}{(6.22)(0.1)}$$

2.95 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β -NADPH at 340 nm

0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will produce 1.0 μmole of ATP from APS and inorganic pyrophosphate per minute at pH 8.0 at 30°C.

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FINAL ASSAY CONCENTRATION:

In a 2.95 ml reaction mix, the final concentrations are 115 mM Tris, 0.58 mM β -nicotinamide adenine dinucleotide phosphate, 2.4 mM magnesium acetate, 34 mM D-glucose, 4 units hexokinase, 2 units glucose 6-phosphate dehydrogenase, 0.3 mM adenosine 5'-phosphosulfate, 0.02 - 0.06 unit adenosine 5'-triphosphate sulfurylase, and 3.4 mM pyrophosphate.

REFERENCES:

Robbins, P.W. (1962) *Methods in Enzymology* V, 964-977

Bergmeyer, H.U., Gawehn, K. and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 2nd ed., Volume I, 473-474

NOTES:

1. This assay is based on the cited references.
2. Hexokinase Unit Definition: One unit will phosphorylate 1.0 μ mole of D-glucose per minute at pH 7.6 at 25°C.
3. Glucose 6-Phosphate Dehydrogenase Unit Definition: One unit will oxidize 1.0 μ mole of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of NADP at pH 7.4 at 25°C.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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