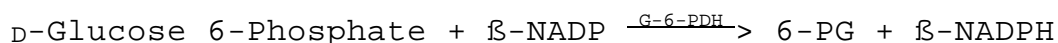
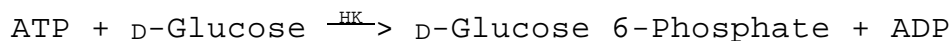
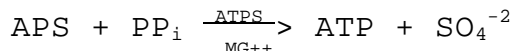


**Enzymatic Assay of ADENOSINE 5'-TRIPHOSPHATE SULFURYLASE  
(EC 2.7.7.4)**

**PRINCIPLE:**



Abbreviations used:

APS = Adenosine 5'-Phosphosulfate

PP<sub>i</sub> = 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<sub>340nm</sub>, 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.)

**Enzymatic Assay of ADENOSINE 5'-TRIPHOSPHATE SULFURYLASE  
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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<sub>i</sub>)  
(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.

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

**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<sub>340nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

|                              |      |      |
|------------------------------|------|------|
| Reagent F (PP <sub>i</sub> ) | 0.10 | 0.10 |
|------------------------------|------|------|

Immediately mix by inversion and record the increase in A<sub>340nm</sub> for approximately 5 minutes. Obtain the r A<sub>340nm</sub>/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

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.

**Enzymatic Assay of ADENOSINE 5'-TRIPHOSPHATE SULFURYLASE  
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**FINAL ASSAY CONCENTRATION:**

In a 2.95 ml reaction mix, the final concentrations are 115 mM Tris, 0.58 mM  $\beta$ -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  $\mu$ 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  $\mu$ 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.

**This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**