

**Enzymatic Assay of NUCLEOSIDE PHOSPHORYLASE
(EC 2.4.2.1)**

PRINCIPLE:

Inosine + Pi $\xrightarrow{\text{Nucleoside Phosphorylase}}$ Hypoxanthine + Ribose-1-PO₄

Hypoxanthine + 2H₂O + 2O₂ $\xrightarrow{\text{Xanthine Oxidase}}$ Uric Acid + 2H₂O₂

Abbreviations:

Pi = Inorganic Phosphate

CONDITIONS: T = 25°C, pH = 7.4, A_{293nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Potassium Phosphate Buffer, pH 7.4 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Prod. No. P-5379. Adjust to pH 7.4 at 25°C with 1 M NaOH.)
- B. 7.5 mM Inosine Solution
(Prepare 5 ml in deionized water using Inosine, Prod. No. I-4125.)
- C. Xanthine Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 10 units/ml of Xanthine Oxidase, Prod. No. X-4500, in ice cold deionized water.)
- D. Nucleoside Phosphorylase Enzyme Solution
(Immediately before use, prepare a solution containing 0.125 units/ml of Nucleoside Phosphorylase in ice cold deionized water.)

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PROCEDURE:

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

		<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.70	2.70	
Reagent B (Inosine)		0.10	0.10
Reagent C (Xanthine Oxidase)	0.10	0.10	

Mix by inversion and equilibrate to 25°C. Monitor the $A_{293\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Nucleoside Phosphorylase)	0.10	-----	
Deionized Water	-----	0.10	

Immediately mix by inversion and record the increase in $A_{293\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{293\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{(r A_{293\text{nm}}/\text{min Test} - r A_{293\text{nm}}/\text{min Blank})}{(12.0) (\text{mg enzyme/ml RM})}$$

12.0 = Millimolar extinction coefficient of Uric Acid
at 293nm
RM = Reaction Mix

UNIT DEFINITION:

One unit will cause the phosphorylation of 1.0 μmole of inosine to hypoxanthine and ribose 1-phosphate per minute at pH 7.4 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.0 ml reaction mix, the final concentrations are 90 mM potassium phosphate, 0.25 mM inosine, 1.0 units of xanthine oxidase and 0.0125 units of nucleoside phosphorylase.

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NOTES:

1. Xanthine Oxidase - One unit will convert 1.0 μ mole of xanthine to uric acid per minute at pH 7.5 at 25°C.
2. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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