SIGMA QUALITY CONTROL TEST

Enzymatic Assay of NUCLEOSIDE PHOSPHORYLASE
(EC 2.4.2.1)

PRINCIPLE:

Inosine + Pi $\rightarrow$ Hypoxanthine + Ribose-1-PO$_4$

Hypoxanthine + 2H$_2$O + 2O$_2$ $\xrightarrow{\text{Xanthine Oxidase}}$ Uric Acid + 2H$_2$O$_2$

Abbreviations:
Pi = Inorganic Phosphate

CONDITIONS: $T = 25^\circ C$, 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.70</td>
<td>2.70</td>
</tr>
<tr>
<td>Reagent B (Inosine)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (Xanthine Oxidase)</td>
<td>0.10</td>
<td>0.10</td>
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</table>

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 \(\text{-----}\) |
| Deionized Water                      | \(\text{-----}\) 0.10 |

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

CALCULATIONS:

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

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

UNIT DEFINITION:

One unit will cause the phosphorolysis of 1.0 $\mu$ mole 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 µmole 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.

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