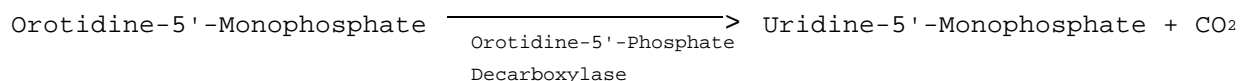
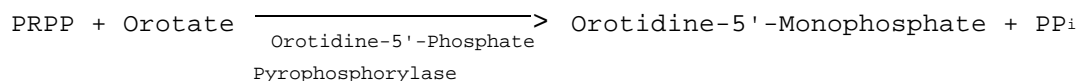


**Determination of the Concentration and Molecular Weight of
PHOSPHORYLRIBOSE-1-PYROPHOSPHATE using OROTATE,
OROTIDINE-5'-PHOSPHATE PYROPHOSPHORYLASE AND
OROTIDINE-5'-PHOSPHATE DECARBOXYLASE**

PRINCIPLE:



Abbreviations Used:

PRPP = Phosphorylribose-1-Pyrophosphate

PP_i = Inorganic Pyrophosphate

CONDITIONS: T = 25°C, pH = 8.0, A_{295nm}, Light path = 1 cm

METHODS: Spectrophotometric

REAGENTS:

- A. 100 mM Tris Buffer, pH 8.0 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 8.0 with 1 M HCl.)
- B. 10 mM Orotic Acid Sodium (Substrate)
(Prepare 50 ml in Reagent A using Orotic Acid Sodium, Prod. No. O-3000. Heat may be necessary to form the solution.)
- C. 100 mM Magnesium Chloride Hexahydrate (MgCl₂•6H₂O)
(Prepare 50 ml in deionized water using Magnesium Chloride Hexahydrate, Prod. No. M-0250.)
- D. Phosphorylribose-1-Pyrophosphate Solution (PRPP)
(Weigh two samples accurately, approximately 2.5 mg, and dissolve in 10 ml deionized water.)

**Determination of the Concentration and Molecular Weight of
PHOSPHORYLRIBOSE-1-PYROPHOSPHATE using OROTATE,
OROTIDINE-5'-PHOSPHATE PYROPHOSPHORYLASE AND
OROTIDINE-5'-PHOSPHATE DECARBOXYLASE**

REAGENTS: (continued)

- E. 25 mM Glycylglycine Buffer, pH 7.0 at 25°C
(Prepare 100 ml in deionized water using Glycylglycine, Prod. No. G-1002. Adjust to pH 7.0 with 1 M HCl.)
- F. Orotidine-5'-Phosphate Pyrophosphorylase and Orotidine-5'-Phosphate Decarboxylase Enzyme Solution
(Prepare approximately 5 units/ml in Reagent E using Prod. No. O-6250. Centrifuge the solution and decant the supernatant.)

PROCEDURE:

Pipette (in milliliters) the following reagent into suitable vials:

	<u>Test</u>	<u>Blank</u>
Deionized Water	-----	1.10
Reagent A (Buffer)	1.65	1.65
Reagent B (Substrate)	0.10	-----
Reagent C (MgCl ₂ •6H ₂ O)	0.05	0.05
Reagent D (PRPP Solution)	1.00	-----

Mix by inversion and obtain the A_{295nm} using a suitable thermostatted spectrophotometer at 25°C. Then add:

Reagent F (Enzyme Solution)	0.20	0.20
-----------------------------	------	------

Mix by inversion and allow the reaction to proceed for 2-3 hours. Upon completion of the reaction record the A_{295nm} and calculate the A_{295nm} and apparent molecular weight.

CALCULATIONS:

$$\Delta A = A_i \times \frac{2.80}{3.00} - A_f ; \quad A_i = \text{initial absorbance}$$

$$A_f = \text{final absorbance}$$

$$\text{micromoles PRPP/weighed sample} = \frac{\Delta A \times 3.00 \times 10}{3.95}$$

**Determination of the Concentration and Molecular Weight of
PHOSPHORYLRIBOSE-1-PYROPHOSPHATE using OROTATE,
OROTIDINE-5'-PHOSPHATE PYROPHOSPHORYLASE AND
OROTIDINE-5'-PHOSPHATE DECARBOXYLASE**

CALCULATIONS: (continued)

3.00 = Total volume of Reaction Mixture
10 = Dilution factor
3.95 = Extinction coefficient of orotate at 295nm

$$\text{Apparent molecular weight} = \frac{\text{mg sample weighed} \times 1000}{\text{micromoles PRPP/weighed sample}}$$

FINAL ASSAY CONCENTRATION:

In a 3.0 ml reaction mix, the final concentrations are
58.3 mM tris, 0.33 mM orotic acid, 1.67 mM MgCl₂•6H₂O,
1.67 mM glycylglycine. and 1.0 unit orotidine-5'-phosphate
pyrophosphorylase.

NOTES:

1. 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.