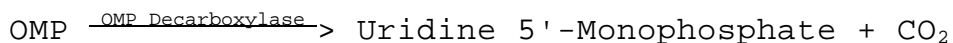
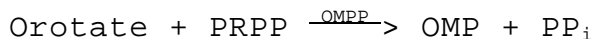


**Enzymatic Assay of OROTIDINE-5'-PHOSPHATE PYROPHOSPHORYLASE
and OROTIDINE-5'-PHOSPHATE DECARBOXYLASE**

PRINCIPLE:



Abbreviations used:

PRPP = 5-Phosphorylribose-1-Pyrophosphate

OMPP = Orotidine-5'-Monophosphate Pyrophosphorylase

OMP = Orotidine 5'-Monophosphate

PP_i = Inorganic Pyrophosphate

OMP Decarboxylase = Orotidine 5'-Monophosphate
Decarboxylase

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

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 25 mM Tris HCl Buffer with 8 mM Magnesium Chloride, pH 8.0 at 25°C
(Prepare 100 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253, and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250. Adjust to pH 8.0 at 25°C with 1 M NaOH.)
- B. 14 mM Orotic Acid Solution (Orotic Acid)
(Prepare 2 ml in deionized water using Orotic Acid, Monosodium Salt, Sigma Prod. No. O-3000. Heating may be required for solubilization.)
- C. 16 mM 5-Phosphorylribose-1-Pyrophosphate Solution (PRPP)
(Prepare 2 ml in deionized water using 5-Phosphorylribose-1-Pyrophosphate, Sodium Salt, Sigma Prod. No. P-8296.)

**Enzymatic Assay of OROTIDINE-5'-PHOSPHATE PYROPHOSPHORYLASE
and OROTIDINE-5'-PHOSPHATE DECARBOXYLASE**

REAGENTS: (continued)

- D. Orotidine-5'-Phosphate Pyrophosphorylase and Orotidine-5'-Phosphate Decarboxylase Enzyme Solution (Immediately before use, prepare a solution containing approximately 26 units/ml of Orotidine-5'-Phosphate Pyrophosphorylase and Orotidine-5'-Phosphate Decarboxylase Mixed Enzymes in cold Reagent A.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.75	2.75
Reagent B (Orotic Acid)		0.05
		0.05
Reagent D (Enzyme Solution)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the $A_{295\text{nm}}$ until constant using a suitably thermostatted spectrophotometer. Then add:

Reagent C (PRPP)	0.10	-----
Deionized Water	-----	0.10

Immediately mix by inversion and record the decrease in A_{295} for approximately 10 minutes. Obtain the $A_{295\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{295\text{nm}}/\text{min Test} - A_{295\text{nm}}/\text{min Blank})(3)(60)(\text{df})}{(3.95)(0.1)}$$

3 = Total volume (in milliliters) of assay
60 = Conversion factor from minutes to hours as per the

Unit Definition

df = Dilution factor

3.95 = Millimolar extinction coefficient¹ of orotic acid

at 295 nm

0.1 = Volume (in milliliter) of enzyme used

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

**Enzymatic Assay of OROTIDINE-5'-PHOSPHATE PYROPHOSPHORYLASE
and OROTIDINE-5'-PHOSPHATE DECARBOXYLASE**

CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will catalyze the phosphorylation of 1.0 μ mole of orotic acid to 5'-OMP, which is then decarboxylated to 5'-UMP in one hour at pH 8.0 at 25°C, in a PRPP system.

FINAL CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 24 mM Tris, 8 mM magnesium chloride, 0.2 mM orotic acid, 0.5 mM 5'-phosphorylribose-1-pyrophosphate, 2.6 units orotidine-5'-phosphate pyrophosphorylase, and orotidine 5'-phosphate decarboxylase mixed enzymes.

REFERENCE:

Möllering, H. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 2nd ed., Volume 4, 1959-1963

NOTES:

1. The millimolar extinction coefficient of orotic acid is described in Möllering, H. (1974).
2. This assay is based on the cited reference.
3. 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.