Enzymatic Assay of OROTIDINE-5'-MONOPHOSPHATE PYROPHOSPHORYLASE
(EC 2.4.2.10)

PRINCIPLE:

OMP + Pyrophosphate $\xrightarrow{OMPP}$ Orotic Acid + PRPP

Abbreviations used:
OMP = Orotidine 5'-Monophosphate
OMPP = Orotidine-5'-Monophosphate Pyrophosphorylase
PRPP = 5-Phosphorylribose-1-Pyrophosphate

CONDITIONS: T = 30°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 30°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250. Adjust to pH 8.0 at 30°C with 1 M HCl.)

B. 35 mM Orotidine 5'-Monophosphate Solution (OMP)
(Prepare 10 ml in deionized water using Orotidine 5'-Monophosphate, Sodium Salt, Sigma Prod. No. O-1376. **PREPARE FRESH.**)

C. 50 mM Pyrophosphate Solution (PPi)
(Prepare 10 ml in deionized water using Tetrasodium Pyrophosphate, Decahydrate, Sigma Prod. No. P-9146. **PREPARE FRESH.**)

D. Orotidine-5'-Monophosphate Pyrophosphorylase Enzyme Solution
(Immediately before use, prepare a solution containing 50 - 100 units/ml of Orotidine 5'-Monophosphate Pyrophosphorylase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.70	2.70
Reagent B (OMP)	0.10	0.10
Reagent D (Enzyme Solution)	0.10	0.10

Mix by inversion and equilibrate to 30°C. Monitor the A_{295nm} until constant using a suitably thermostatted spectrophotometer. Then add:

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

Immediately mix by inversion and record the increase in A_{295nm} for approximately 5 minutes. Obtain the ΔA_{295nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{(\Delta A_{295nm}/\text{min Test} - \Delta A_{295nm}/\text{min Blank})\ (60)}{(3.5)\ (\text{mg enzyme/ml RM})}$$

60 = Conversion factor from minutes to hours as per the Unit Definition
3.5 = Millimolar extinction coefficient of orotic acid at 295 nm
RM = Reaction Mix

UNIT DEFINITION:

One unit will convert 1.0 µmole of orotidine 5'-monophosphate to orotic acid in one hour at pH 8.0 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 23 mM Tris, 7 mM magnesium chloride, 1.2 mM orotidine 5'-monophosphate, 1.7 mM pyrophosphate and 5 - 10 units

orotidine-5'-monophosphate pyrophosphorylase.

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REFERENCE:

Flaks, J. G. (1963) *Methods in Enzymology* **VI**, 148-152.

NOTES:

1. This assay is a modification of the procedure described in the cited reference.

2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.