Enzymatic Assay of OROTIDINE-5'-MONOPHOSPHATE DECARBOXYLASE (EC 4.1.1.23)

PRINCIPLE:

Orotidine 5'-Monophosphate $\xrightarrow{\text{OMP Decarboxylase}}$ UMP + CO_2
Abbreviations used:
OMP Decarboxylase = Orotidine 5'-Monophosphate Decarboxylase
UMP = Uridine 5'-Monophosphate

CONDITIONS: T = 30°C, pH = 8.0, A_{295nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 30 mM Tris HCl Buffer, pH 8.0 at 30°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 30°C with 1 M HCl.)

B. 75 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

C. 18 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**.)

D. Orotidine-5'-Monophosphate Decarboxylase Enzyme Solution
(Immediately before use, prepare a solution containing 30 - 60 units/ml of Orotidine 5'-Monophosphate Decarboxylase in cold deionized water.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.50	2.50
Reagent B ($MgCl_2$)	0.30	0.30
Reagent C (OMP)	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 D (Enzyme Solution)	0.10	------

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{295nm}/\text{min Test} - \Delta A_{295nm}/\text{min Blank})\ (60)\ (3)}{(0.50)(0.1)}$$

3 = Total volume (in milliliters) of assay
60 = Conversion from minutes to hours (Unit definition)
0.50 = The delta millimolar extinction coefficient between OMP and UMP at 295 nm
0.1 = Volume (in milliliters) of enzyme used

UNIT DEFINITION:

One unit will convert 1.0 µmole of orotidine 5'-monophosphate to uridine 5'-monophosphate per hour at pH 8.0 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 25 mM Tris, 7.5 mM magnesium chloride, 0.6 mM orotidine 5'-monophosphate and 3 - 6 units orotidine 5'-monophosphate decarboxylase.

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REFERENCES:

Lieberman, I., Kornberg, A., and Simms, E.S. (1955) *J. Biol. Chem.* **215**, 403-415.

NOTES:

1. This assay is based on the cited reference.

2. 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.