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

PRINCIPLE:

Orotidine 5'-Monophosphate (OMP) Decarboxylase → UMP + CO₂
Abbreviations used:
OMP Decarboxylase = Orotidine 5'-Monophosphate Decarboxylase
UMP = Uridine 5'-Monophosphate

CONDITIONS:  T = 30°C, pH = 8.0, \( A_{295\text{nm}} \), 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 (MgCl₂)
(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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Reagent B (MgCl₂)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent C (OMP)</td>
<td>0.10</td>
<td>0.10</td>
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</table>

Mix by inversion and equilibrate to 30°C. Monitor the A₂₉₅nm until constant, using a suitably thermostatted spectrophotometer. Then add:

<p>| | | |</p>
<table>
<thead>
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<tbody>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in A₂₉₅nm for approximately 5 minutes. Obtain the r A₂₉₅nm/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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:


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.