Enzymatic Assay of PROLIDASE  
(EC 3.4.13.9)

PRINCIPLE:

\[
\text{Glycyl-}L\text{-Proline} + H_2O \xrightarrow{\text{Prolidase}} \text{Glycine} + L\text{-Proline}
\]

CONDITIONS:  \( T = 40^\circ C, \ pH = 8.0, \ A_{242\text{nm}}, \ \text{Light path} = 1 \ \text{cm} \)

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A.  50 mM Tris HCl Buffer, pH 8.0 at 40°C  
(Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 8.0 at 40°C with 1 M HCl.)

B.  30 mM Glutathione Solution (GSH)  
(Prepare 5 ml in deionized water using Glutathione, Fresh Acid, Reduced Form, Prod. No. G-4251. PREPARE FRESH.)

C.  200 mM Manganese Chloride Solution (MnCl₂)  
(Prepare 10 ml in deionized water using Manganese Chloride, Tetrahydrate, Prod. No. M-3634.)

D.  25 mM Glycyl-\(L\)-Proline Solution (Gly-Pro)  
(Prepare 30 ml in Reagent A using Gly-Pro, Prod. No. G-3002. Adjust to pH 8.0 at 40°C with 1 M HCl or 1 M NaOH, if necessary.)

E.  Prolidase Enzyme Solution  
(Immediately before use prepare a solution containing approximately 5 mg/ml of Prolidase in cold Reagent A.)
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PROCEDURE:

Prepare an activated enzyme (activation mix with enzyme) by pipetting (in milliliters) the following reagents into a suitable container in the order specified:

<table>
<thead>
<tr>
<th>Reagent A (Buffer)</th>
<th>2.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (MnCl(_2))</td>
<td>0.40</td>
</tr>
<tr>
<td>Reagent B (GSH)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Mix and incubate at 40°C for 20, 25, and 30 minutes. Immediately after completing the activation step, pipette (in milliliters) the following reagents into suitable quartz cuvettes:

<table>
<thead>
<tr>
<th>Reagent D (Gly-Pr)</th>
<th>2.70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (MnCl(_2))</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 40°C. Monitor the \(A_{242\text{nm}}\) until constant, using a suitably thermostatted spectrophotometer. Then add:

| Activation Mix with Enzyme\(^2\) | 0.50 |
| Activation Mix without Enzyme | ------ |

Immediately mix by inversion and record the decrease in \(A_{242\text{nm}}\) for approximately 5 minutes. Obtain the \(\Delta A_{242\text{nm}}\) using the maximum linear rate\(^3\) for both the Test and Blank.

CALCULATION:

\[
\text{Units/ml enzyme} = \frac{(\Delta A_{242\text{nm}}/\text{min Test} - \Delta A_{242\text{nm}}/\text{min Blank})(3.1)(3.4)}{(0.0254)(0.5)(0.2)}
\]

3.1 = Volume (in milliliters) of activation mix  
3.4 = Volume (in milliliters) of reaction mix  
0.0254 = Millimolar extinction coefficient for Glycyl-L-proline at 242 nm  
0.5 = Volume (in milliliter) of activated enzyme used in assay  
0.2 = Volume (in milliliter) of enzyme used in activation mix
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CALCULATION: (continued)

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

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of glycyl-\(L\)-proline per minute at pH 8.0 at 40°C.

FINAL ASSAY CONCENTRATION:

In a 3.40 ml reaction mix, the final concentrations are 5.7 mM Tris, 16 mM manganese chloride, 0.14 mM glutathione, 20 mM glycyl-\(L\)-proline and 0.16 mg prolidase.

REFERENCE:


NOTES:

1. Do not adjust the pH of the Activation Mixture.
2. Use the activated enzyme from all three time points. The time point which provides the largest \(A_{242\text{nm}}\) should be used in the calculation.
3. The \(A_{242\text{nm}}/\text{min}\) should be between 0.03 - 0.05.
4. This assay is based on the cited reference.
5. 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.