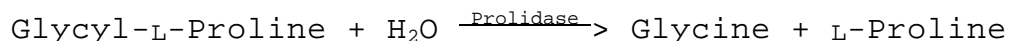


**Enzymatic Assay of PROLIDASE
(EC 3.4.13.9)**

PRINCIPLE:



CONDITIONS: T = 40°C, pH = 8.0, A_{242nm}, Light path = 1 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.)

**Enzymatic Assay of PROLIDASE
(EC 3.4.13.9)**

PROCEDURE:

Prepare an activated enzyme (activation mix with enzyme) by pipetting (in milliliters) the following reagents into a suitable container in the order specified¹:

Reagent A (Buffer)	2.40
Reagent C (MnCl ₂)	0.40
Reagent B (GSH)	0.10
Reagent E (Enzyme Solution)	0.20

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:

	<u>Test</u>	<u>Blank</u>
Reagent D (Gly-Pro)	2.70	2.70
Reagent C (MnCl ₂)	0.20	0.20

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

Activation Mix with Enzyme ²	0.50	-----
Activation Mix without Enzyme	-----	0.50

Immediately mix by inversion and record the decrease in A_{242nm} for approximately 5 minutes. Obtain the ΔA_{242nm} using the maximum linear rate³ 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

**Enzymatic Assay of PROLIDASE
(EC 3.4.13.9)**

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 μ mole 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:

Davis, N. C. and Smith, E. L., (1957) *J. Biol. Chem.* **224**, 261-275

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.