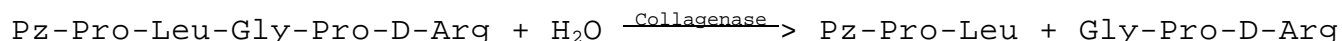


**Enzymatic Assay of COLLAGENASE
Pz-Pro-Leu-Gly-Pro-D-Arg as the Substrate
(EC 3.4.24.3)**

PRINCIPLE:



Abbreviations used:

Pz = 4-Phenylazobenzoyloxycarbonyl

CONDITIONS: T = 37°C, pH = 7.1, A_{320nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 100 mM Tris HCl Buffer, pH 7.1 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.1 at 37°C with 1 M HCl.)
- B. Methanol
(Use Methanol, Absolute, Sigma Stock No. 17-5.)
- C. 1.3 mM Pz-Pro-Leu-Gly-Pro-D-Arg Substrate Solution
(Prepare by dissolving 10 mg of Pz-Pro-Leu-Gly-Pro-D-Arg, Sigma Prod. No. P-8383, in 0.2 ml of Reagent B. Bring to a total volume of 10 ml with Reagent A.)
- D. 100 mM Calcium Chloride Solution (CaCl₂)
(Prepare 100 ml in deionized water using Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881.)
- E. 25 mM Citric Acid Solution (Citric Acid)
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate, Sigma Prod. No. C-7129.)
- F. Ethyl Acetate (ETOAC)
(Use Ethyl Acetate, Sigma Stock No. 27,052-0.)
- G. Sodium Sulfate (Na₂SO₄)
(Use Sodium Sulfate, Anhydrous, Sigma Prod. No. S-6264.)

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REAGENTS: (continued)

- H. 20 mM Calcium Chloride Solution (Enz Dil)
(Prepare 100 ml in deionized water using Reagent D.)
- I. Collagenase Enzyme Solution
(Immediately before use, prepare a solution containing
200 - 500 units/ml of Collagenase in cold Reagent H.)

PROCEDURE:

Step 1:

Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Blank</u>
Reagent C (Substrate Solution)	2.00	2.00
Reagent D (CaCl ₂)	0.40	0.40

Mix by swirling and equilibrate to 37°C. Then add:

Reagent I (Enzyme Solution)	0.10	-----
Reagent H (Enz Dil)	-----	0.10

Immediately mix by swirling and incubate for exactly 15 minutes at 37°C.

Step 2:

Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Blank</u>
Test Mixture (from Step 1)	0.50	-----
Blank Mixture (from Step 1)	-----	0.50
Reagent E (Citric Acid)		1.00
		1.00
Reagent F (ETOAC)	5.00	5.00

Mix by shaking for 15 seconds. Remove the ethyl acetate phase (upper layer) from both the Test and Blank and place into separate tubes, each containing 300 mg of Reagent G (Na₂SO₄). Mix by shaking and filter through Whatman #54 filter paper. Transfer the solutions to suitable cuvettes and record the A_{320nm} for both the Test and Blank using a suitable spectrophotometer.

**Enzymatic Assay of COLLAGENASE
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CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{A}_{320\text{nm}} \text{ Test} - \text{A}_{320\text{nm}} \text{ Blank})(2.5)(\text{df})}{(0.042)(0.5)(0.1)}$$

2.5 = Total volume (in milliliters) of assay in Step 1

df = Dilution factor

0.042 = Extinction coefficient of 0.01 micromole of
Pz-Pro-Leu in 5 ml of ethyl acetate

0.5 = Volume (in milliliter) of reaction mixture in Step 1
used in Step 2

0.1 = Volume (in milliliter) of enzyme used in Step 1

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

UNITS DEFINITION:

One unit will liberate 0.01 μ mole of Pz-Pro-Leu from Pz-Pro-Leu-Gly-Pro-D-Arg (P-8383) in 15 minutes at pH 7.1 at 37°C.

FINAL CONCENTRATIONS:

In a 2.50 ml reaction mix, the final concentrations are 1 mM Pz-Pro-Leu-Gly-Pro-D-Arg, 1.6% (v/v) methanol, 78 mM Tris, 17 mM calcium chloride, and 20 - 50 units collagenase.

REFERENCE:

Wünsch, E. and Heidrich, H.-G. (1963) *Zeitschrift für Physiologische Chemie* **333**, 149-151

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.