



Product Information

SIGMA QUALITY CONTROL TEST PROCEDURE

**Enzymatic Assay of COLLAGENASE using
N-(3-[2-FURYL]ACRYLOYL)-LEU-GLY-PRO-ALA (FALGPA)
as the Substrate
(EC 3.4.24.3)**

PRINCIPLE:

FALGPA $\xrightarrow{\text{Collagenase}}$ FAL + Gly-Pro-Ala

Abbreviations used:

FALGPA = N-(3-[2-Furyl]Acryloyl)-Leu-Gly-Pro-Ala

FAL = N-(3-[2-Furyl]Acryloyl)-Leu

CONDITIONS: T = 25°C, pH 7.5, $A_{345\text{nm}}^1$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Tricine Buffer with 10 mM Calcium Chloride and 400 mM Sodium Chloride, pH 7.5 at 25°C (Prepare 500 ml in deionized water using Tricine, Sigma Prod. No. T0377, Calcium Chloride, Dihydrate, Sigma Prod. No. C3881, and Sodium Chloride, Sigma Prod. No. S9625. Adjust to pH 7.5 at 25°C with 1 M NaOH.)
- B. 1.0 mM N-(3-[2-Furyl]Acryloyl)-Leu-Gly-Pro-Ala Solution (FALGPA) (Prepare 50 ml in Reagent A using N-(3-[2-Furyl]Acryloyl)-Leu-Gly-Pro-Ala, Sigma Prod. No. F5135. Approximately 30 minutes of stirring is required for this product to dissolve completely. Adjust to pH 7.5 at 25°C with either 1 M NaOH or 1 M HCl.)
- C. Collagenase Enzyme Solution (Immediately before use, prepare a solution containing 2 units/ml of Collagenase in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (FALGPA)	2.90	2.90
Deionized Water	-----	0.10

Mix by inversion and equilibrate to 25°C. Monitor the $A_{345\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent C (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the decrease in $A_{345\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{345\text{nm}}/\text{minute}$ by using the maximum linear rate for both the Test and Blank.

CALCULATION:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{345\text{nm}}/\text{min Test} - \Delta A_{345\text{nm}}/\text{min Blank})(3)(\text{df})}{(0.53)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

0.53 = Millimolar extinction coefficient of FALGPA at 345 nm²

0.1 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit hydrolyzes 1.0 μmole of furylacryloyl-Leu-Gly-Pro-Ala (FALGPA, F5135) per minute at 25°C at pH 7.5 in the presence of calcium ions.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 48 mM tricine, 9.7 mM calcium chloride, 387 mM sodium chloride, 0.97 mM N-(3-[2-furyl]acryloyl)-Leu-Gly-Pro-Ala, and 0.20 unit collagenase.

REFERENCE:

Van Wart, H.E., and Steinbrink, D. R. (1981) *Analytical Biochemistry* **113**, 356-365

NOTES:

1. It is critical that the wavelength is set at exactly 345 nm.
2. This value has been determined experimentally by Sigma. It is directly related to the maximum decrease in absorbance per millimole of FALGPA at 345 nm.
3. This assay is based on the cited reference.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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