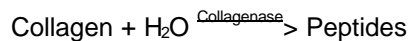


Enzymatic Assay of COLLAGENASE¹
(EC 3.4.24.3)
Collagen Digestion Assay

PRINCIPLE:



CONDITIONS: T = 37°C, pH = 7.4, A_{570nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 50 mM TES Buffer with 0.36 mM Calcium Chloride, pH 7.4 at 37°C
(Prepare 1000 ml in deionized water using TES Free Acid, Sigma Prod. No. T-1375, and Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881. Adjust the pH to 7.4 at 37°C with 1 M NaOH.)
- B. Collagen Type I
(Use Collagen, Type I, Sigma Prod. No. C-9879. Different lots of collagen will produce varying amounts of enzyme activity when used as a substrate for collagenase.)
- C. Collagenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.05 - 0.1 mg/ml Collagenase in Buffer A.)
- D. Ethylene Glycol Monoethyl Ether
(Use Ethylene Glycol Monoethyl Ether, Sigma Prod. No. E-2632.)
- E. 4% (w/v) Ninhydrin Solution
(Prepare 100 ml in Reagent D, using Ninhydrin, Sigma Prod. No. N-4876.)
- F. 200 mM Citrate Buffer with 0.16% (w/v) Stannous Chloride, pH 5.0 at 25°C
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Anhydrous, Sigma Prod. No. C-0759. Adjust to pH 5.0 at 25°C with 5 M NaOH; then add the Stannous Chloride, Anhydrous, Sigma Prod. No. S-2752.)

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REAGENTS: (continued)

- G. 50% (v/v) Isopropanol Solution (Isopropanol)
(Prepare 100 ml in deionized water using Isopropanol, Anhydrous, Sigma Stock No. 405-7.)
- H. Ninhydrin Color Reagent (NCR)
(Immediately before use, combine equal volumes of Reagent E and Reagent F.)
- I. 10 mM Hydrochloric Acid Solution
(Prepare 50 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)
- K. 4.0 mM L-Leucine Standard Solution (Std Soln)
(Prepare 20 ml in Reagent I using L-Leucine, Sigma Prod. No. L-8000. **PREPARE FRESH.**)

PROCEDURE:

Weigh the following reagent into suitable containers:

	Test	Blank
Reagent B (Collagen)	25.00 mg	25.00 mg

Then add (in milliliters) the following reagent:

Reagent A (Buffer)	5.00	5.00
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Incubate the vials at 37°C until equilibrated. Then add:

Reagent A (Buffer)	-----	0.10
Reagent C (Enzyme Solution)	0.10	-----

Mix well and incubate at 37°C. Swirl the containers for 10 - 15 seconds at 1.5 and 3.5 hours. After 5 hours, filter the contents of the containers through a Whatman #54 filter paper or a 0.8 µm syringe filter into clean containers. Use the filtrates for color development.

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COLOR DEVELOPMENT:

Standard Curve:

Prepare a standard curve by pipetting the following reagents (in milliliters) into suitable containers.

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std Blank</u>
Rgnt K (Std Soln)	0.05	0.10	0.15	.20	0.00
Deionized Water	0.15	0.10	0.05	0.00	0.20
Reagent H (NCR)	2.00	2.00	2.00	2.00	2.00

Sample:

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

	<u>Test</u>	<u>Blank</u>
Test Filtrate	0.20	-----
Blank Filtrate	-----	0.20
Reagent H (NCR)	2.00	2.00

Mix well and place vented caps on each container. Place the containers in a boiling water bath for 30 minutes. Remove the containers and allow to cool to room temperature. Add 10 ml of Reagent G (Isopropanol) to each container. Mix well and transfer the container contents to suitable cuvettes. Determine the absorbance at 570 nm for each of the containers using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

$$\ddot{A}_{570\text{nm}} \text{ Standard} = A_{570\text{nm}} \text{ Standard} - A_{570\text{nm}} \text{ Standard Blank}$$

Prepare a standard curve by plotting the $\ddot{A}_{570\text{nm}}$ of the L-Leucine Standard Solution versus micromoles of L-Leucine.

Sample Determination:

$$\ddot{A}_{570\text{nm}} \text{ Sample} = A_{570\text{nm}} \text{ Test} - A_{570\text{nm}} \text{ Test Blank}$$

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CALCULATIONS: (continued)

Determine the imoles of L-Leucine equivalents liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\text{imoles of L-Leucine equivalents liberated}) (5.1) (\text{df})}{(0.2) (0.1)}$$

df = Dilution factor

5.1 = Total volume (in milliliters) of Assay

0.2 = Total volume (in milliliter) of sample used in Colorimetric Determination

0.1 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit liberates peptides from collagen (C-9879) equivalent in ninhydrin color to 1.0 μ mole of leucine in 5 hours at pH 7.4 at 37°C in the presence of calcium ions.

FINAL ASSAY CONCENTRATION:

In a 5.10 ml reaction mix, the final concentrations are 50 mM TES, 0.36 mM calcium chloride, 25 mg collagen and 0.005 - 0.01 mg collagenase.

REFERENCES:

Moore, S. and Stein, W.H. (1948) *J. Biol. Chem.* **176**, 367-388

Mandl, I., MacLennan, J.D., Howes, E.L., DeBellis, R.H., and Sohler, A. (1953) *Journal of Clinical Investigation* **32**, 1323-1329

NOTES:

This assay procedure is not to be used to assay Collagenase, Sigma Prod. Nos. C-1913, C-7926, C-8051, C-8176, and C-8301.

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NOTES: (continued)

2. This assay is based on the cited references.
3. 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.