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ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Suitability Assay for COLLAGEN

PRINCIPLE:

Collagen + $H_2O \xrightarrow{\text{Collagenase}}$ Peptides

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
(Different types 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 0.1 mg/ml Collagenase, Sigma Prod. No. C-0130, 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 1 M NaOH. Then add the Stannous Chloride, Anhydrous, Sigma Prod. No. S-2752.)

REAGENTS: (continued)

G. 50% (v/v) 1-Propanol Solution
(Prepare 100 ml in deionized water using 1-Propanol, Sigma Stock No. 29,328-8.)

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. 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

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 vials.

		Std 1	Std 2	Std 3	Std 4	Std Blank
Reagent K (Std Soln)	0.05	0.10	0.15	0.20	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 vials:

	Test	Blank
Test Filtrate	0.20	------
Blank Filtrate	------	0.20
Reagent H (NCR)	2.00	2.00

Mix well and place vented caps on each vial. Place the vials in a boiling water bath for 20 minutes. Remove the vials and allow to cool to room temperature. Add 10 ml of Reagent G (50% 1-Propanol) to each vial. Mix well and transfer the vial contents to suitable cuvettes. Determine the absorbance at 570 nm for each of the vials using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

ΔA_{570nm} Standard = A_{570nm} Standard - A_{570nm} Standard blank

Prepare a standard curve by plotting the ΔA_{570nm} of the L-Leucine Standard Solution versus micromoles of L-Leucine.

Sample Determination:

ΔA_{570nm} Sample = A_{570nm} Test - A_{570nm} Sample blank

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CALCULATIONS: (continued)

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

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

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 equivalent in ninhydrin color to 1.0 µmole of leucine in 5 hours at pH 7.4 at 37°C in the presence of calcium ions.

SPECIFICATION:

Suitable for use as a substrate for Collagenase.

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
0.01 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

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NOTES:

1. This assay is based on the cited references.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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