SIGMA QUALITY CONTROL TEST PROCEDURE

Suitability Assay for COLLAGEN

PRINCIPLE:

Collagen + H₂O \text{Collagenase} \rightarrow \text{Peptides}

CONDITIONS: T = 37°C, pH = 7.4, A₅₇₀nm, 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.)
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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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (Collagen)</td>
<td>25.00 mg</td>
<td>25.00 mg</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Reagent K (Std Soln)</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>0.05</td>
<td>0.10</td>
<td>0.15</td>
<td>0.20</td>
<td>0.00</td>
</tr>
<tr>
<td>Reagent H (NCR)</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Sample:

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Filtrate</td>
<td>0.20</td>
</tr>
<tr>
<td>Blank Filtrate</td>
<td>-----</td>
</tr>
<tr>
<td>Reagent H (NCR)</td>
<td>2.00</td>
</tr>
</tbody>
</table>

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:

\[ \Delta A_{570\text{nm}} \text{ Standard} = A_{570\text{nm}} \text{ Standard} - A_{570\text{nm}} \text{ Standard blank} \]

Prepare a standard curve by plotting the \[ \Delta A_{570\text{nm}} \] of the L-Leucine Standard Solution versus micromoles of L-Leucine.

Sample Determination:

\[ \Delta A_{570\text{nm}} \text{ Sample} = A_{570\text{nm}} \text{ Test} - A_{570\text{nm}} \text{ Sample blank} \]
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CALCULATIONS: (continued)

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

\[
\text{Units/ml enzyme} = \frac{\text{(μmoles of L-Leucine equivalents liberated)} \times (5.1) \times (\text{df})}{(0.2) \times (0.1)}
\]

\( \text{df} \) = 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:


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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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