SIGMA QUALITY CONTROL TEST PROCEDURE

Suitability Assay for AZO DYE IMPREGNATED COLLAGEN

PRINCIPLE:

\[
\text{Azocoll} + \text{H}_2\text{O} \xrightarrow{\text{Protease}} \text{Colored Reaction Products}
\]

Abbreviation used:

\text{Azocoll} = \text{Azo Dye Impregnated Collagen}

CONDITIONS: \( T = 37^\circ \text{C}, \text{pH} = 7.0, A_{520 \text{nm}}, \text{Light path} = 1 \text{ cm} \)

METHOD: Colorimetric

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 7.0 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Sigma Prod. No. P-5379. Adjust to pH 7.8 at 37°C with 1 M KOH.)

B. Azo Dye Impregnated Collagen (Azocoll)
(Use Azo Dye Impregnated Collagen, Sigma Prod. No. A-4341.)

C. Protease Enzyme Solution (Protease) Step 1
(Immediately before use, prepare a solution containing 0.09 - 0.13 unit/ml of Protease, Sigma Prod. No. P-5380, in cold Reagent A. Store at 0 - 4°C.)

D. Protease Enzyme Solution (Protease) Step 2
(Immediately before use, prepare a solution containing 10.0 - 12.00 units/ml of Protease, Sigma Prod. No. P-5380, in cold Reagent A. Store at 0 - 4°C.)

PROCEDURE:

Weigh (in milligrams) the following reagents into 4 dram vials:

<table>
<thead>
<tr>
<th>Reagent B (Azocoll)</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Test 4</th>
<th>Test 5</th>
<th>Test 6</th>
<th>Blank</th>
</tr>
</thead>
</table>

Then add (in milliliters):

| Reagent A (Buffer) | 5.08 | 5.06 | 5.04 | 5.02 | 5.00 | 5.00 | 5.00 |
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PROCEDURE: (continued)

Equilibrate to 37°C. Then add at 1 minute time intervals:

<table>
<thead>
<tr>
<th></th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Test 4</th>
<th>Test 5</th>
<th>Test 6</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Protease)</td>
<td>0.02</td>
<td>0.04</td>
<td>0.06</td>
<td>0.08</td>
<td>0.10</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Reagent D (Protease)</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

Incubate at 37°C for exactly 15 minutes in a shaking water bath (60 cycles/minute).

Remove the vials and immediately filter the suspension through 0.45 micron syringe filters. Transfer the filtered solutions to suitable cuvettes and record the $A_{520}$nm using a suitable spectrophotometer.

CALCULATIONS:

Sample Curve:

$$\Delta A_{520\text{nm}} \text{ Sample} = A_{520\text{nm}} \text{ Test} - A_{520\text{nm}} \text{ Test Blank}$$

Plot the $\Delta A_{520\text{nm}}$ Sample vs units of protease per assay.

SPECIFICATIONS:

The sample is suitable if it has similar results compared to that of a control. The plot of absorbance versus units of protease should be nonlinear. The $\Delta A_{520\text{nm}}$ of Test 6 >1.0.

FINAL ASSAY CONCENTRATION:

In a 5.00 ml reaction mix, the final concentrations are 100 mM phosphate, 0.5% (w/v) azo dye impregnated collagen, and 0.009 - 0.013 unit protease.

REFERENCE:

Suitability Assay for AZO DYE IMPREGNATED COLLAGEN

NOTES:

1. This assay is based on the cited reference.

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

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