Suitability Assay for KERATIN AZURE
as a Substrate for Proteinase K

PRINCIPLE:

\[
\text{Proteinase K} \quad \text{Keratin Azure} + H_2O \rightarrow \text{Colored Reaction Product}
\]

CONDITIONS: \(T = 37^\circ\text{C}, \text{pH} = 7.5, A_{595\text{nm}}, \text{Light path} = 1 \text{ cm}\)

METHOD: Colorimetric

REAGENTS:

A. 50 mM Sodium Phosphate Buffer, pH 7.5 at 37°C
   (Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous, Sigma Prod. No. S-0876. Adjust to pH 7.5 at 37°C with 1 M HCl.)

B. Keratin Azure
   (Use Keratin Azure, Sigma Prod. No. K-8500.)

C. Proteinase K Enzyme Solution
   (Immediately before use, prepare a solution containing 100 units/ml of Proteinase K, Sigma Prod. No. P-6556, in cold deionized water.)

PROCEDURE:

Weigh (in milligrams) the following reagents into suitable containers:

<table>
<thead>
<tr>
<th></th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Test 4</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (Keratin Azure)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Test 4</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.40</td>
<td>0.30</td>
<td>0.20</td>
<td>0.10</td>
<td>0.50</td>
</tr>
</tbody>
</table>
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PROCEDURE: (continued)

Mix by swirling and equilibrate at 37°C for 10 minutes in a water bath. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Test 4</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (Keratin Azure)</td>
<td>0.10</td>
<td>0.20</td>
<td>0.30</td>
<td>0.40</td>
<td>------</td>
</tr>
</tbody>
</table>

Mix by swirling and incubate at 37°C for exactly 1 hour on a metabolic shaker where constant mixing can be maintained.

Centrifuge the solutions for 10 minutes and transfer the supernatants to suitable cuvettes. Record the $A_{595\text{nm}}$ for both the Tests and Blanks with a suitable spectrophotometer.

CALCULATIONS:

$\Delta A_{595\text{nm}}$ Test = $A_{595\text{nm}}$ Test - $A_{595\text{nm}}$ Blank

$\Delta A_{595\text{nm}}$/hour/mg enzyme = $\frac{\Delta A_{595\text{nm}}$ Test}{mg enzyme/RM}

RM = Reaction Mixture

SPECIFICATION:

Compare the $\Delta A_{595\text{nm}}$/hour/mg enzyme of the test to that of a control sample. These values should be similar.

FINAL ASSAY CONCENTRATION:

In a 4.50 ml reaction mix, the final concentrations are 44 mM sodium phosphate, 0.44% (w/v) keratin azure, and 10 - 40 units proteinase K.

REFERENCE:

Wainwright, M. (1982), Experientia 38, 243-244

NOTES:

1. This assay is based on the cited reference.

2. 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.