Enzymatic Assay of ELASTIN–CONGO RED

PRINCIPLE:
Insoluble Elastin–Congo Red + H₂O \text{Elastase}\rightarrow \text{Soluble Hydrolysis Products}

CONDITIONS: T = 37°C, pH = 8.8, A₄₉₅nm, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 200 mM Tris HCl Buffer, pH 8.8 at 37°C
   (Prepare 100 ml in deionized water using Trizma Base, Adjust to pH 8.8 at 37°C with 1 M HCl.)

B. Elastin–Congo Red Substrate (El-CR)
   (Use Elastin–Congo Red.)

C. Elastase Enzyme Solution
   (Immediately before use, prepare a solution containing approximately 1500 - 1800 units/ml of Elastase, in cold Reagent A.)

PROCEDURE:

Step 1: Standard Curve

Weigh (in milligrams) the following reagent:

<table>
<thead>
<tr>
<th></th>
<th>Std 1</th>
<th>Blank 1</th>
<th>Std 2</th>
<th>Blank 2</th>
<th>Std 3</th>
<th>Blank 3</th>
<th>Std 4</th>
<th>Blank 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (EL-CR)</td>
<td>5.00</td>
<td>5.00</td>
<td>10.00</td>
<td>10.00</td>
<td>15.00</td>
<td>15.00</td>
<td>20.00</td>
<td>20.00</td>
</tr>
</tbody>
</table>

Then add (in milliliters):

<table>
<thead>
<tr>
<th></th>
<th>Std 1</th>
<th>Blank 1</th>
<th>Std 2</th>
<th>Blank 2</th>
<th>Std 3</th>
<th>Blank 3</th>
<th>Std 4</th>
<th>Blank 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>6.00</td>
<td>6.01</td>
<td>6.00</td>
<td>6.01</td>
<td>6.00</td>
<td>6.01</td>
<td>6.00</td>
<td>6.01</td>
</tr>
</tbody>
</table>

Mix and equilibrate to 37°C. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Std 1</th>
<th>Blank 1</th>
<th>Std 2</th>
<th>Blank 2</th>
<th>Std 3</th>
<th>Blank 3</th>
<th>Std 4</th>
<th>Blank 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Enzyme)</td>
<td>0.01</td>
<td>----</td>
<td>0.01</td>
<td>----</td>
<td>0.01</td>
<td>----</td>
<td>0.01</td>
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</tbody>
</table>
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PROCEDURE: (continued)

Mix by swirling and place all the containers in a suitably thermostatted metabolic shaker. Incubate the Standards and Standard Blanks at 37°C for 12 - 16 hours. Filter the solutions through 0.8 µm syringe filter. Record the $A_{590nm}$ for the Standards and Standard Blanks using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

\[ r \ A_{495nm} \ Standard = A_{495nm} \ Std - A_{495nm} \ Std \ Blank \]

Prepare a standard curve by plotting the $A_{495nm}$ of the Standards vs milligrams of solubilized Elastin–Congo Red.

\[ r \ A_{495nm} / mg \ Elastin-Congo \ Red = \frac{r \ A_{495nm}}{mg \ Elastin-Congo \ Red} \]

Compare the result of the Test to that of a control sample. The activity should be similar.

FINAL ASSAY CONCENTRATION:

In a 6.01 ml reaction mix, the final concentrations are 200 mM Tris, 5 - 20 mg elastin–congo red and 15 - 18 units elastase.

REFERENCE:


NOTES:

1. The Metabolic shaker should be adjusted to approximately 60 shake cycles/minute.

2. If the filtrate is hazy, it can be centrifuged to remove the haziness.

3. Elastase Unit Definition: One unit will solubilize 1 mg of elastin in 20 minutes at pH 8.8 at 37°C.

4. This assay is based on the cited reference.