

Enzymatic Assay of ELASTIN-CONGO RED

PRINCIPLE:

Insoluble Elastin-Congo Red + H₂O $\xrightarrow{\text{Elastase}}$ Soluble Hydrolysis Products

CONDITIONS: T = 37°C, pH = 8.8, A_{495nm}, 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, Sigma Prod. No. T-1503. Adjust to pH 8.8 at 37°C with 1 M HCl.)
- B. Elastin-Congo Red Substrate (El-CR)
(Use Elastin-Congo Red, Sigma Prod. No. E-0502.)
- C. Elastase Enzyme Solution
(Immediately before use, prepare a solution containing approximately 1500 - 1800 units/ml of Elastase, Sigma Prod. No. E-0127, in cold Reagent A.)

PROCEDURE:

Step 1: Standard Curve

Weigh (in milligrams) the following reagent:

	<u>Std 1</u>	<u>Blank 1</u>	<u>Std 2</u>	<u>Blank 2</u>	<u>Std 3</u>	<u>Blank 3</u>	<u>Std 4</u>	<u>Blank 4</u>
Reagent B (EL-CR)	5.00	5.00	10.00	10.00	15.00	15.00	20.00	20.00

Then add (in milliliters):

Reagent A (Buffer)	6.00	6.01	6.00	6.01	6.00	6.01	6.00	6.01
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Mix and equilibrate to 37°C. Then add:

Reagent C (Enzyme)	0.01	----	0.01	----	0.01	----	0.01	----
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PROCEDURE: (continued)

Mix by swirling and place all the containers in a suitably thermostatted metabolic shaker.¹ Incubate the Standards and Standard Blanks at 37°C for 12 - 16 hours. Filter the solutions through 0.8 µm syringe filter.² Record the A_{590nm} for the Standards and Standard Blanks using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

$$r A_{495nm} \text{ Standard} = A_{495nm} \text{ Std} - A_{495nm} \text{ Std Blank}$$

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

$$r A_{495/mg} \text{ Elastin-Congo Red} = \frac{r A_{495nm}}{\text{mg Elastin-Congo Red}}$$

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:

Shotton, D.M. (1970) *Methods in Enzymology*, XIX, 113-119

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

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This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.