

Enzymatic Assay of ELASTASE¹ (EC 3.4.21.36)

PRINCIPLE:

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

CONDITIONS: T = 37°C, pH = 8.8, A_{590nm}, 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-Orcein Substrate (EI-Or)
(Use Sigma Prod. No. E-1500.)
- 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 reagents:

	<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-OR)	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
--------------------	------	------	------	------	------	------	------	------

Mix and equilibrate to 37°C. Then add:

Reagent C (Enzyme)	0.01	---	0.01	---	0.01	---	0.01	---
--------------------	------	-----	------	-----	------	-----	------	-----

Enzymatic Assay of ELASTASE¹
(EC 3.4.21.36)

PROCEDURE: (continued)

Mix by swirling and place all the containers in a suitably thermostatted metabolic shaker.² Incubate the Standards and Standard Blanks at 37°C for 12 - 16 hours. Filter the solutions through 0.8 µm syringe filter.³ Record the A_{590nm} for the Standards and Standard Blanks using a suitable spectrophotometer.

Step 2: Samples

Weigh (in milligrams) the following reagents:

	<u>Test 1</u>	<u>Test 2</u>	<u>Test 3</u>	<u>Test Blank</u>
Reagent B (El-Or)	50.00	50.00	50.00	50.00

Then add (in milliliters):

Reagent A (Buffer)	6.00	5.995	5.99	6.01
--------------------	------	-------	------	------

Mix and equilibrate to 37°C. Then add:

Reagent C (Enzyme)	0.01	0.015	0.02	-----
--------------------	------	-------	------	-------

Mix by swirling and place all the containers in a suitably thermostatted metabolic shaker.² Incubate the Tests and Test Blank at 37°C for 20 minutes. Filter the solution through 0.8 µm syringe filters.³ Record the A_{590nm} for the Tests and Test Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

$$\Delta A_{590nm} \text{ Standard} = A_{590nm} \text{ Std} - A_{590nm} \text{ Std Blank}$$

Prepare a standard curve by plotting the ΔA_{590nm} of the Standards vs milligrams of solubilized elastin-orcein⁴.

Sample Determination:

$$\Delta A_{590nm} \text{ Sample} = A_{590nm} \text{ Test} - A_{590nm} \text{ Blank}$$

Determine the mg of solubilized elastin-orcein using the Standard Curve.

Enzymatic Assay of ELASTASE¹
(EC 3.4.21.36)

CALCULATIONS: (continued)

$$\text{Units/ml enzyme} = \frac{(\text{mg of elastin solubilized}) (\text{df})}{0.01}$$

df = Dilution Factor

0.01 = Volume (in milliliters) of enzyme used

$$\text{Units/ml solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/ml protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will solubilize 1 mg of elastin in 20 minutes at pH 8.8 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 6.01 ml reaction mix, the final concentrations are 200 mM Tris, 50 mg elastin-orcein and 15 - 36 units elastase.

REFERENCE:

Sachar, L. A., Winter, K.K., Sicher, N., and Frankel, S., (1955) *Proceedings of the Society for Experimental Biology and Medicine* **90**, 323-326

NOTES:

1. This assay procedure is not to be used to assay Elastase, Leukocyte, Sigma Prod. No. E-8140.
2. The Metabolic shaker should be adjusted to approximately 60 shake cycles/minute.
3. If the filtrate is hazy, it can be centrifuged to remove the haziness.
4. Due to the variance of Elastin-Orcein lots, the standard curve may not be consistent. The amount of enzyme utilized in the assay is not affected by this variance.

Enzymatic Assay of ELASTASE¹
(EC 3.4.21.36)

NOTES: (continued)

5. This assay is based on the cited reference.
6. 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.