Catalog Number: 100617, 100619

Elastase

Molecular Weight: 25,500

CAS # : 9004-06-2

Source: *Porcine Pancreas*

Activity: Approx. 50 u/mg protein

Form: 2X crystallized. Aqueous suspension containing 0.01% NaN_3 as preservative. 75 units per mg of protein on the substrate elestin-porcein. One unit will solubilize 1 mg of elastin in 20 minutes at pH 8.8 and 37°C. [6] Greater that 4 units per mg of protein on the substrate Suc-$(Al)_3$-pNA. One will hydrolyze 1 mmole per minute at pH 8.3 and 25°C.

Preparation: Two times crystallized from the euglobin fraction of porcine pancreas by the method of Lewis et al. [7]. Does not contain trypsin or chymotrypsin.

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Description: Hydrolyzes peptide bonds, especially those adjacent to neutral amino acids.

Elastase is prepared from porcine pancreas. It is lyophilized and water soluble. Elastase is chromatographically prepared by the method of Narayanan and Anwar. In the method, 2X crystalline elastase is adsorbed on a column of DEAE-Sephadex A50 to separate elastase and non-specific protein components. The elastase component is further purified by chromatography on a column of CM-Cellulose using a sodium chloride gradient to elute the elastase. The latter is dialyzed until chloride-free and then lyophilized.

The preparation is free of tryptic and chymotrypic activities as shown by assays using p-toluene-sulfonyl-L-arginine methyl ester (TAME) as substrate for trypsin activity and benzoyl-L-tyrosine ethyl ester (BTEE) as substrate for chymotrypsin activity.

Note: During its preparation the elastase is held below a pH of 5.5 for greater than 24 hours.

Inhibitors: DFP, elastinal, a-2-macroglobulin.

Typical Procedure for Suspending the Substrate Powders in Buffer:

Materials Required:

1. Substrate Powder, e.g., Elastin-Rhodamine, 200-400 Mesh.
2. Buffers

    0.2 M Tris pH 8.8 containing 0.01% Triton X-100 and 0.01% Sodium Azide.
    0.2 M Tris pH 8.8 containing 0.01% Sodium Azide.

3. Whatman No. 41 paper or equivalent.
4. Powder Funnel.
5. Magnetic Stirrer.

Procedure:

Add the substrate (20 mg/ml) to gently stirring buffer containing Triton X-100 and stir until all particles are wetted. Wash the substrate on No. 41 paper with buffer (not containing Triton X-100) until the filtrate is colorless. Resuspend the substrate in buffer (not containing Triton X-100) to 20 mg/ml. NOTE: Always maintain a layer of buffer over the substrate while washing. Do not allow the substrate to filter to dryness or clumping of the substrate particles may occur.

Typical Procedure for Determination of Elastolytic Activity by Spectrophotometry:

The following procedure is an example using Elastin-Rhodamine, 200-400 mesh. Other substrates may likewise be used. Each substrate has a distinct absorbency after being solubilized by elastase. The absorbency of the soluble peptides of dyed elastins follows:

Elastin-Rhodamine, 550 nm
Elastin-Fluorescein, 495 nm
Elastin-Orcein, 570 nm
Elastin-Congo Red, 485 nm
Elastin-Remazol, 595 nm

Step 1. Known quantities of substrate are totally solubilized by elastase. The optical density per mg of substrate is determined.
Step 2. Known quantities of elastase are incubated with substrate. The quantity of substrate solubilized per mg of elastase (i.e., specific activity) is determined. The elastolytic activity of an unknown can be determined in comparison to the activity of elastase.

Materials Required:

1. Substrate Suspension: 20 mg/ml in 0.2 M Tris-HCl pH 8.8, 0.01% Sodium Azide.
2. Buffer: 0.2 M Tris pH 8.8 containing 0.01% Sodium Azide.
3. Elastase: 2X Crystallized or Chromatographically Purified.
4. Conical Flask, 10-25 ml size or Test Tube.
5. Dubnoff type incubator: equilibrated at 37°C.
6. Magnetic stirrer.
7. Ice Bath.
8. Filter paper, Whatman No. 41 or equivalent.

Step 1. Determination of Optical Density of Solubilized Substrate

Examine the following protocol and pipet buffer then elastase into 10 ml flasks. Keeping the substrate in suspension by stirring magnetically, add aliquots to the flasks using a 1.0 ml blow-out pipet.

NOTE: The elastase aliquot should contain 20-30 units activity. This is an excess amount which is necessary to completely solubilize the substrate within 30-60 minutes.

Flask (No.)	Buffer (ml)	Elastase (ml)	Substrate (ml)	Substrate (mg)	Observed O.D.	O.D. per mg Substrate (Calculated)
1 (Blank)	2.90	0.10	0	0	0	0
2	2.65	0.10	0.25	5.0		
3	2.40	0.10	0.50	10.0		
4	2.15	0.10	0.75	15.0		

5	1.90	0.10	1.00	20.0

Stopper the flasks and incubate at 37°C with 40-60 excursions per minute until all of the substrate has been solubilized (30-60 minutes). Bring the volume to 10 ml with buffer and read the O.D. in 10 nm cuvetts against the blank. A secondary dilution must be prepared in order that all readings fall within the range of the spectrophotometer. Record each O.D. per mg (multiplied times the dilution factor) and calculate the O.D. per mg of substrate. The O.D. per mg should be nearly constant (± 5%). A plot of O.D. vs mg of substrate should be a straight line.

Step 2. Determination of Units Elastolytic Activity

Examine the following protocol and pipet buffer then elastase then substrate into 10 ml flasks. Keep the substrate in suspension when delivering aliquots. The elastase concentration should be 0.2 mg per ml in buffer.

Extinction Coefficient: $E^{1\%}_{280\ nm} = 19.5$

$A_{280} \times 0.51 = mg/ml$

Flask (No.)	Buffer (ml)	Elastase (ml)	Substrate (mg)	Substrate (ml)	Observed O.D.	mg Substrate Solubilized (Calculated)
1 (Blank)	2.0	0	0	1.0	0	0
2	1.9	0.1	0.02	1.0		
3	1.8	0.2	0.04	1.0		
4	1.7	0.3	0.06	1.0		
5	1.6	0.4	0.08	1.0		
6	1.5	0.5	0.10	1.0		

Stopper the flasks and incubate with shaking at 37°C for 20 minutes. Submerge the flasks in ice and bring the volume to 10 ml with cold buffer. Rapidly filter through No. 41 paper into cuvetts and read the O.D. of the filtrates against the blank. Record observed O.D. Calculate mg of substrate solubilized by dividing observed O.D. by the constant O.D. per mg previously determined in Step 1.

Calculate mg substrate solubilized per mg of elastase. This final calculation gives specific activity. The most widely used unit definition for specific elastolytic activity is: One unit will solubilize 1 mg of elastin in 20 minutes at pH 8.8 and 37°C.

The elastolytic activity of an unknown may be determined by substituting aliquots of the unknown for elastase in the above procedure. The relative amounts of buffer and unknown can be varied; however, the final volume of the incubate (3 ml), the pH (8.8) and molarity (0.2 M Tris) must be constant.

Typical Procedure for the Determination of Elastase Activity of Porcine Pancreatic Elastase using Suc-Al-Al-Al-pNA as substrate:

Materials Required:

1. Tris buffer: 0.1 M Tris pH 8.3 at 25°C containing 0.01% Sodium Azide. Dissolve 6.75 g Tris-HCl, 8.14 g Tris-base and 0.10 g Sodium Azide in 900 ml water. Determine the pH at 25°C. Titrate if necessary to pH 8.3 with 0.1 M HCl or 0.1 M Sodium hydroxide. Dilute to 1000 ml with water.
2. NaOAc-NaCl buffer: 0.05 M NaOAc pH 5.0 containing 0.1 M NaCl and 0.01% Sodium Azide. Combine 14.8 ml of 0.2 M HOAc and 35.2 ml of 0.2 M NaOAc and 100 ml of 0.2 M NaCl. Add 0.1 g Sodium Azide and bring to 200 ml with water. Titrate to pH 5 at 25°C.
3. Substrate solution, 4.43 mM: Dissolve 50 mg N-Suc-Al-Al-Al-pNA in 0.5 ml of 1-methyl-2-pyrrolidinone,

bring to 25 ml with 2 mM HOAc. (NOTE: An acetic acid solution of N-Suc-Al-Al-Al-pNA is more stable than a solution in Tris buffers.)

4. Elastase Solution: Dissolve 1.0 mg per ml in the NaOAc-NaCl buffer. Prepare a secondary dilution of 0.10 mg per ml in the same buffer. Keep both solutions cold in an ice bath.

Procedure:

1. Adjust the spectrophotometer to 410 nm and cell temperature to 25°C.
2. Equilibrate 2.8 ml of Tris buffer and 0.2 ml of substrate solution to 25°C in the cell.
3. Add 0.005 ml of the 0.10 mg per ml elastase solution, mix and determine the rate increase in absorbency at 1.0 minute intervals. The rate increase should be approximately 0.025-0.040 D410 nm per minute.

Calculation of Specific Activity:

$E^{1\%}_{280}$ = 19.5 for porcine pancreatic elastase

1 mg/ml = A_{280} x 0.51

Vol = 3.005 ml

A = 410 nm

T = 25°C

Light Path = 1.0 cm

8.8 mM extinction coefficient of pNA at 410 nm

Availability:

Catalog Number	Description	Size
100617	Elastase, 2X crystallized, Aqueous suspension with 0.01% sodium azide as preservative, Activity is greater than 50 units/mg protein	10 mg 25 mg 50 mg 100 mg
100619	Elastase, lyophilized powder, Activity is not less than 95 units/mg protein	5 mg 10 mg 20 mg 100 mg

References:

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