ELASTIN PRODUCTS COMPANY, INC
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TECHNICAL DATA SHEET

ELASTASE EC 3.4.21.36

No. ES438

Date of Preparation:

Lot No_____
Data Location Book 12B page 71

Assay	Units/ mg Protein	mg Protein/ml
Elastase	153.6	25.0
Trypsin	.013	.0021
Chymotrypsin	.041	.0055

Re-Assay Date 14 November 2008

SOURCE. Porcine Pancreas.

SPECIFICATIONS.
2 X crystallized, MW=25,500. Aqueous suspension containing 0.01% NaN_3 as preservative. Store 5ºC. Stable 9-12 months at 5°C. DO NOT FREEZE.

120-145 units per mg of protein on the substrate elastin-orcein
One unit will solubilize 1 mg of elastin in 20 minutes at pH 8.8 and 37ºC (1).
See assay procedure A.

Greater than 4 units per mg of protein on the substrate N-Suc-Ala-Ala-Ala-pNA (EPC No. NS945). One unit will hydrolyze 1µmole per minute at pH 8.3 and 25ºC.
See assay procedure B.
PREPARATION.
Two times crystallized fron the euglobin fraction of porcine pancreas by our propriatory method based on Lewis et al., (2).
Contaminants; less than 0.002% Trypsin and less than 0.15% Chymotrypsin.

(1) Sacher, L.A., Winter, K.K., Sicher, N. and Frankel, S., Proc. Soc. Exp. Med. Biol., 90, 323, (1955).
(2) Lewis, U.J., Williams, D.E. and Brink, N.G., J. Biol. Chem, 22, 705, (1956).

Assay Procedure A

Procedure for Determination of Elastolytic Activity Using Elastin

The following procedure is an example using Elastin=Rhodamine, 200-400 mesh. Other substrates may likewise be used. Each substrate has a distinct absorbancy after being solubilized by elastase. The absorbance 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

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Elastin-Remazol, 595 nm

The procedure closely follows published methods (9,10,11,35,36).

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; 20mg/ml in 0.2 M Tris-HCl pH 8.8, 0.01% NaN_3.
2. Buffer; 0.2 M Tris pH 8.8 containing 0.01% NaN_3.
3. Elastase; 2X crystallized or chromatographically purified.
4. Conical Flasks, 10-25 ml size or Test Tubes.
5. Dubnoff type incubator; equilibrated at 37°C.
6. Magnetic stirrer.
7. Ice Bath.
8. Filter paper, Whatman No 41 or equivalent.

*See page 5.

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 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 solublized (30-60 minutes). Bring the volume to 10 ml with buffer and read the O.D. in 10 mm cuvetts against the blank. A secondary dilution must be prepared in order that all readings fall within 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

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substrate in suspension when delivering aliquots. The elastase concentration should be 0.2 mg per ml in buffer.

Extinction coefficient: ∈ 1%, 280 nm = 19.5
A_{280} x .51 = mg/ml

Flask (No.)	Buffer (ml)	Elastase (ml)	Substrate (ml)	Substrate (mg)	Observed O.D.	O.D. per mg Substrate (Calculated)
1(Blank)	2.0	0	0	1.0	0	0
2	1.9	0.1	.02	1.0	--------	--------
3	1.8	0.2	.04	1.0	--------	--------
4	1.7	0.3	.06	1.0	--------	--------
5	1.6	0.4	.08	1.0	--------	--------
6	1.5	0.5	.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.
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$$\frac{\text{Units}}{\text{mg}} = \frac{\text{mg Substrate Solubilized}}{\text{mg Elastase}} = \frac{\text{Observed O.D.}}{\text{O.D./mg Substrate}} \Big/ \text{mg Elastase}$$

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 37ºC. 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.

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Procedure for Suspending Elastin 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% Trixton* X-100
   and 0.01% NaN_3.
   0.2 M Tris pH 8.8 containing 0.01% Nan_3
3. Whatman No. 41 paper or equivalent.
4. Powder Funnel.
5. Magnetic Stirrer.

Procedure.

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

Storage. Store at 5ºC.

*Triton is a registered trademark of Rohm and Hass Company.

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ASSAY PROCEDURE B

Determination of Porcine Pancreatic Elastase Activity

Assay with Suc-Ala-Ala-Ala-pNA (EPC No. NS945) as substrate (44).

Materials Required.

1. Tris buffer; 0.1 M Tris pH 8.3 at 25ºC containing 0.01% NaN_3. Dissolve 6.75 g Tris-HCl, 8.14 G Tris base and 0.10 g NaN_3 in 900 ml H_2O. Determine pH at 25ºC. Titrate if necessary to pH 8.3 with 0.1 M HCl or 0.1 M NaOH. Dilute to 1000 ml with H_2O.
2. NaOAc-NaCl buffer; 0.05 M NaOAc pH 5, containing 0.1 M NaCl and 0.01% NaN_3. Combine 14.8 ml of 0.2 M HAc and 35.2 ml of 0.2 M NaOAc and 100 ml 0.2 M NaCl. Add 0.10 g NaN_3 and bring to 200 ml with H_2O. Titrate to pH 5 at 25ºC.
3. Substrate solution; 2.5 mM in 0.1 M Tris pH 8.3. Utilizing a 25 mg vial of N-Suc-Ala-Ala-Ala-pNA (EPC No NS945), dissolve the contents with 22 ml of tris buffer. (Note: Use about 10 ml of the buffer for 5 flushes of the substrate vial.) Dissolve with stirring. Store at 5ºC.
4. Elastase solution; dissolve 1.0 mg per ml in the NaOAc-NaCl buffer. Prepare a secondary solution 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 25ºC.
2. Equilibrate 2.5 ml of Tris buffer and 0.5 ml of substrate solution to 25º in the cell.
3. Add 0.005 ml of the 0.10 mg ml elastase solution, mix and determine the rate increase in absorbency at 1 minute intervals. The rate increase should be ca. 0.025 – 0.040 Δ_{410} nm per minute.

Calculation of Specific Activity.

$\in$, 1%, 280 = 19.5 for porcine pancreas elastase

mg/ml = A_{280} x 0.51

Vol = 3.005 ml A=410 nm T=25ºC Light Path=1.0 cm
8.8=mM extinction coefficient of pNA at 410 nm

$$\frac{\text{Units}}{\text{mg}} = \frac{\Delta A_{410} \text{ x } 3.005 \text{ ml}}{8.8 \text{ x } 0.0005 \text{ mg}}$$

DETERMINATION OF PORCINE PANCREATIC CHYMOTRYPSIN ACTIVITY
Assay with N-Succinyl-Ala-Ala-Pro-Phe-pNA (EPC No. SG554).

Materials Required.

1. Buffer; 0.10 M Tris pH 8.3 prepared at 25°C. Dissolve 6.14 g Tris-HCl, 7.40 g Tris-base and 0.10 g NaN_3 in 900 ml H_2O. Determine the pH and titrate if necessary with 0.1 M HCl or 0.1 M NaOH to pH 8.3 at 25°C. Bring to 1000 ml with H_2O.

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2. Substrate solution; 1.2 mM. Prepare by dissolving 2 mg N-Succinyl-Ala-Ala-Pro-Phe-pNA in 2.6 ml 1-methyl-2-pyrrolidone or DMSO. Store 5°C.

3. Enzyme solution. Dissolve 0.1 mg/ml in 0.05 M NaOAc pH 5 containing 0.1 M NaCl. Store 5°C.

PROCEDURE

1. Adjust the spectrophotometer to 410 nm and the cell temperatures to 25°C.

2. Dispense 2.4 ml of buffer solution in a cell. Add 0.6 ml substrate solution. Mix well, equilibrate to25°C in the cell.

3. Add 0.10 ml of enzyme solution and determine the rate increase in absorbency at 1 minute intervals. The rate of increase should be linear for up to 5 minutes.

CALCULATION OF SPECIFIC ACTIVITY

Vol - 3.10 ml A = 410 T = 25°C Light Path = 1 cm

8.8 = mM extinction coefficient of pNA at 410 nm.

$$\frac{\text{Units}}{\text{mg}} = \frac{\Delta A \text{ /min} \times 3.10 \text{ ml}}{8.8 \times 0.01 \text{ mg}}$$

DETERMINATION OF PORCINE PANCREATIC TRYPSIN
Assay with N-Benzoyl-Phe-Val-Arg-pNA (EPC No. NB32) as substrate.

Materials required.

1. Buffer; 0.1M Tris pH 8.3 prepared at 25°C. Mix 0.1 M Tris-HCl into 0.1 M Tris-base until the pH = 8.3 at 25°C.

2. Buffer; 0.05 NaOAc pH 5.0. Mix 0.05 M HAc into 0.05 M NaOAc until pH = 5.0 at 25°C.

3. Substrate solution; 1.0 mM. Prepare by dissolving 68.0 mg N-Benzoyl-Phe-Val-Arg-pNA (EPC No. NB32) in 1 ml of 1-methyl-2-Pyrrolidinone. Bring to 100 ml with the tris buffer.

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4. Enzyme solution. Dissolve to 0.1 mg per ml in 0.05 M NaOAc pH 5. Maintain at 5°C.

Procedure.

1. Adjust the spectrophotometer to 410 nm and the cell temperature to 25°C.

2. Pipette 2.70 ml of buffer (pH 8.3 Tris) into the cell. Add 0.3 ml substrate solution. Mix, equilibrate to 25°C in the cell.

3. Add 0.01 ml enzyme solution and determine the increase in absorbency per minute from the initial reaction.

Calculation of Specific Activity

Vol = 3.01 ml A = 410 nm T = 25°C Light Path = 1 cm

$\in$ 1 mM pNA = 8.8

$$\frac{\text{Units}}{\text{mg}} = \frac{(\Delta A_{410}/\text{min})\ (3.01\text{ml})}{8.8 \times 0.01\ \text{mg}}$$