

Catalog Number: 100921, 100924, 102566

## Papain

**Molecular Weight:** 23,000

**CAS # :** 9001-73-4

**Physical Description:** White to off white powder

**Description:** Papain is a sulfhydryl protease from *Carica papaya* latex. (A second protease, chymopapain, and a lysozyme have also been isolated from this same source.) Since native crystalline papain is quite unreactive until acted upon by mild reducing agents such as cysteine or cyanide, it may exist as a zymogen (Brocklehurst and Kierstan 1973). For a general review, see Liener (1974). Barrett and Buttle (1985), Polgár (1984) and Brocklehurst and Salih (1983) report on the classification of papaya latex proteinases. Papain has wide specificity. In her review, Arnon (1970) has indicated that it will degrade most protein substrates more extensively than the pancreatic proteases. It is also an esterase. Papain has been reviewed by Smith and Kimmel (1960). It has been reported by Sluyterman and Wijdenes (1972a) that the action of papain on leucine methyl ester produces an insoluble polyleucine peptide. The finding of Thomas (1956) that papain breaks down the intercellular matrix of cartilage (see also McCluskey and Thomas 1958), led to its further study as a chondromucoproteinase (Smith et al. 1962).

Proteolytic enzymes are widely used in cell isolation. With some tissues papain has proved less damaging and more effective than other proteases. Lam (1972) found that of the enzymes used for dissociating turtle retina, papain produced the least trauma. Intact single photoreceptor cells have also been isolated from adult salamander retina with papain (Bader et al. 1978, Townes-Anderson et al. 1985). Huettner and Baughman (1986) described a method using papain to obtain high yields of viable, morphologically intact cortical neurons from postnatal rats. Finkbeiner and Stevens (1988) applied the Huettner and Baughman method to the dissociation of postnatal rat hippocampus. Papain is used with fetal as well as postnatal brain regions to provide maximal dissociation and viability of neurons.

**Composition:** Papain is a single peptide chain of 211 residues folded into two parts that form a cleft (Dreuth et al. 1968). A three-dimensional structure has been indicated by Wolthers et al. (1970). The molecule has one free SH group which is functional (Smith et al. 1975; Shipton et al. 1975). According to Alecio et al. (1974) there are seven subsites each capable of accommodating a single amino acid residue of a peptide substrate. See also Glick and Brubacker (1974). Other reports on molecular information and its relation to activity are as follows: Fink and Gwyn (1974), Lewis and Shafer (1974), Akalski et al. (1973), Allen and Lowe (1973), Brocklehurst and Little (1973), Mole and Horton (1973a, b, and c), Banks and Shafer (1972), Brocklehurst et al. (1972), Campbell and Kaiser (1971, 1972), Sluyterman and Wijdenes (1972b), Hinkle and Kirsch (1971a and b) Jori et al. (1971), Lowe and Yuthavong (1971a and b) and Steiner (1971).

**Optimum pH:** 6.0 - 7.0.

**Extinction coefficient:** 25.0 (Mitchel et al. 1970).

**Isoelectric point:** pH 9.6 (Sluyterman and DeGraff 1972).

**Activators:** Papain is activated by cysteine, sulfide, sulfite, etc. It is enhanced when heavy metal binding agents such as EDTA are also present. Kirschenbaum (1971) indicated that N-bromosuccinimide enhances the activity. Hall et al. (1972) report on the affects of acridine dyes.

**Inhibitors:** Substances which react with sulfhydryl groups including heavy metals, carbonyl reagents (Moriyama 1967). Westerik and Wolfenden (1972) have studied aldehydes as papain inhibitors and Sluyterman and Wijdenes (1973) report on benzoylamidoacetonitrile as an inhibitor. See Shapira and Arnon (1967a and b) on antibody inhibitors. Papain may be inactivated by H<sub>2</sub>O<sub>2</sub> generated by  $^{60}\text{Co}$ -irradiation of H<sub>2</sub>O- the active SH group being oxidized

to sulfenic acid. (Lin et al 1975). See also Allison and Swain (1973). Specific inhibitors are AEBSF, antipain, cystatin, E-64, leupeptin, PMSF, TLCK and TPCK.

**Stability:** Papain as a crystalline suspension is stable at 5°C for 6-12 months. Stabilizing agents are EDTA, cysteine and dimercaptopropanol.

To enhance stability as well as solubility it may be advantageous to convert crystalline papain to its mercury derivative (Brubacher and Bender 1966).

### Assay

**Method:** A titrimetric determination of the acid produced during the hydrolysis of benzoyl-L-arginine ethyl ester (BAEE). One unit will hydrolyze one micromole of benzoyl-L-arginine ethyl ester per minute at 25°C and pH 6.2 under the specified conditions.

### Reagents

*Enzyme diluent (Activation buffer):* Prepare fresh daily by mixing the following:

0.01 M EDTA	10 ml
0.06 M b-Mercaptoethanol	0.1 ml
0.05 M Cysteine·HCl	10 ml
Reagent grade water	70 ml

*Substrate solution:* Prepare fresh daily by mixing the following:

0.058 M BAEE	15.0 ml
0.01 M EDTA	0.8 ml
0.05 M Cysteine·HCl	0.8 ml

Adjust pH to 6.2 and dilute to a final volume of 21 ml with reagent grade water.

*Titrant:* 0.01-0.02 N NaOH, standardized

### Enzyme

Activate enzyme by dissolving in enzyme diluent to a concentration of 0.05-0.1 mg/ml. Under these conditions activation is complete within 30 minutes.

### Determination of protein concentration

$$\text{Papain: mg protein/ml} = A_{280} \times 0.4$$

### Procedure

The reaction can be measured with either an automatic titrator or a laboratory pH meter. The titration vessel should be maintained at 25°C.

Pipette the following into the titration vessel at 25°C:

Substrate solution	5.0 ml
3.0 M NaCl	5.0 ml
Reagent grade water	5.0 ml

At zero time add 0.1 ml of appropriately diluted enzyme and adjust the pH to 6.2. Record the amount of standardized NaOH added per minute to maintain the pH at 6.2 after a constant rate is achieved.

### Calculation

ml base added/minute x normality x 1000

U/mg =

mg enzyme in reaction buffer

**Technical note:** Mercuripapain must be activated before use. Mercury is removed from the enzyme in activation buffer. After 30 minutes in this solution, the enzyme is completely activated and the mercury has been chelated. The mercuripapain suspension contains no free mercury. The product has been extensively dialyzed prior to packaging.

### Availability:

Catalog Number	Description	Sizes
100921	Papain, 2X crystallized, suspension in 0.05 M sodium acetate. Activity is approximately 10 - 20 units/mg protein	25 mg
		50 mg
		100 mg
		500 mg
		1 g
100924	Papain, 2X crystallized, A suspension in 0.05 M sodium acetate. Activity is approximately 15 - 40 units/mg protein	5 g
		25 mg
		100 mg
		250 mg
		500 mg
102566	Papain, Technical powder. This is a crude, dried papaya latex which is not purified.	1 g
		5 g
		100 g
		250 g
		500 g
		1 kg

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