

## Product Information

### Papain from papaya latex

Catalog Number **P4762**  
Storage Temperature  $-20\text{ }^{\circ}\text{C}$

CAS RN 9001-73-4  
EC 3.4.22.2  
Synonyms: Papainase, papaya peptidase I

#### Product Description

Papain is a cysteine protease with wide specificity, cleaving peptide bonds of basic amino acids, leucine, or glycine. It also hydrolyzes esters and amides. Papain will digest most protein substrates more extensively than the pancreatic proteases.

Papain consists of a single polypeptide chain with three disulfide bridges and a sulfhydryl group necessary for activity of the enzyme. The specificity of cleavage of the X-Y bond is: where X is a nonspecific amino acid, but arginine and lysine are preferred; and phenylalanine-X-Y bond where residues following phenylalanine are preferred; Y is a nonspecific amino acid residue.<sup>1</sup>

Papain is commonly used in cell isolation procedures where it has proven more efficient and less destructive than other proteases on certain tissues. For example, papain has been used to isolate viable, morphologically intact, cortical neurons from postnatal rats.<sup>2</sup> This papain preparation (Catalog No. P4762) has been used for the isolation of smooth muscle cells.<sup>3,4</sup> Papain was found to significantly increase the yield of viable smooth muscle cells while not affecting cell sensitivity to stimulants.<sup>5</sup>

Limited papain digestion has proven useful for structural studies of enzymes and other proteins.<sup>6-8</sup> Papain is used in red cell serology to modify the red cell surface to enhance or destroy the reactivity of many red cell antigens as an adjunct to grouping, antibody screening, or antibody identification procedures. Papain has also been shown to be useful in platelet serology.<sup>9</sup>

Papain has also been used in the enzymatic synthesis of amino acids, peptides, and other molecules.<sup>10-13</sup>

Papain is used routinely for the preparation of Fab fragments from IgG. The protease cleaves the antibodies into two Fab fragments, which recognize the antigen specifically with their variable region, and one Fc fragment.<sup>14</sup> IgM may also be digested with papain resulting in high yields of homogeneous Fab preparations.<sup>15</sup>

Molecular mass:<sup>16</sup> 23,406 Da (amino acid sequence)

Optimal pH: 6.0–7.0

Isoelectric point (pI):<sup>17,18</sup> 8.75; 9.55

Spectral properties:

$\lambda_{\text{max}}$ :<sup>19</sup> 278 nm

Extinction coefficient ( $E^{1\%}$ ):<sup>19</sup> 25

Extinction coefficient ( $E^{\text{mM}}$ ):<sup>20</sup> 57.6 (280 nm)

Inhibitors:

Antipain (Catalog No. A6191)

Cystamine (Catalog No. C121509)

Chymostatin (Catalog No. C7268)

Cystatin (Catalog No. C8917)

3,4-Dichloroisocoumarin (Catalog No. D7910)

E-64 (Catalog No. E3132)

Ebselen (Catalog No. E3520)

Gly-Gly-Tyr-Arg (Catalog No. G5386)

Leupeptin (Catalog No. L2023)

$\alpha_2$ -Macroglobulin (Catalog No. M6159)

Potassium halide salts at low pH have also been reported to inhibit papain.<sup>18</sup>

Substrates:

pGlu-Phe-Leu *p*-nitroanilide (Catalog No. P3169)

Gelatin (Catalog No. G2500 or G1890)

This papain product is purified from papaya latex, crystallized two times, and supplied as a lyophilized powder containing sodium acetate.

Activity:  $\geq 10$  units/mg protein

Unit Definition: One unit will hydrolyze 1.0  $\mu$ mole of N- $\alpha$ -benzoyl-L-arginine ethyl ester (BAEE) per minute at pH 6.2 at 25 °C.

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

Papain is soluble in water (10 mg/ml) to form a stock solution. Immediately prior to use, the enzyme is typically diluted in buffer containing ~5 mM L-cysteine.

### Storage/Stability

The powder as supplied should be stored at  $-20$  °C.

Although papain solutions have good temperature stability, the solution stability is pH dependent. Papain solutions are unstable under acidic conditions (*i.e.*, at pH values below 2.8, there is a significant decrease in activity). For the active enzyme in solution, the loss in activity is 1–2% per day, probably as a result of autolysis and/or oxidation.

Papain solutions are stable to several denaturing agents (*i.e.*, full activity is maintained after recrystallization in 70% methanol and in 8 M urea solutions). However, there is a significant loss in activity when papain is exposed to 10% trichloroacetic acid or to 6 M guanidine hydrochloride.

A suspension of papain crystals in sodium chloride solution can be kept at 2–8 °C for months without detectable loss in activity. Stabilizing agents include EDTA, cysteine, and dimercaptopropanol.<sup>21</sup>

### References

1. Carrey, E.A., in *Protein Structure: A Practical Approach*, 2nd ed. (Creighton, T. E., ed.), IRL Press, (New York, NY: 1997), pp 117-144.
2. Huettner, J.E., and Baughman, R.W., *J. Neurosci.*, **6**, 3044-3060 (1986).
3. Kinoshita, K., *et al.*, *Am. J. Physiol. Gastrointest. Liver Physiol.*, **285**, G483-G493 (2003).
4. Driska, S.P., *et al.*, *J. Appl. Physiol.*, **86**, 427-435 (1999).
5. Hasegawa, M., *et al.*, *Nippon Heikatsukin Gakkai Zasshi*, **23**, 35-46 (1987).
6. Margossian, S.S., and Lowey, S., *J. Mol. Biol.*, **74**, 301-311 (1973).
7. Margossian, S.S., and Lowey, S., *J. Mol. Biol.*, **74**, 313-330 (1973).
8. Shiozaki, K., and Yanagida, M., *Mol. Cell. Biol.*, **11**, 6093-6102 (1991).
9. Lown, J.A., and Dale, B.J., *Immunohematol.*, **11**, 140-142 (1995).
10. Xiang, H., *et al.*, *Amino Acids*, **27**, 101-105 (2004).
11. Rajesh, M., *et al.*, *J. Agric. Food Chem.*, **51**, 2461-2467 (2003).
12. Fukuoka, T., *et al.*, *Biomacromolecules*, **3**, 768-774 (2002).
13. Burton, S.G., *et al.*, *Nature Biotech.*, **20**, 37-45 (2002).
14. *Antibodies: A Laboratory Manual*, Harlow, E., and Lane, D., eds., Cold Spring Harbor Laboratory (Cold Spring Harbor, NY: 1988) pp. 628-629.
15. Newkirk, M.M., *et al.*, *Hybridoma*, **6**, 453-460 (1987).
16. Mitchel, R.E., *et al.*, *J. Biol. Chem.*, **245**, 3485-3492 (1970).
17. Smith, E.L., *et al.*, *J. Biol. Chem.*, **207**, 533-549 (1954).
18. Sluyterman, L.A., and de Graaf, M.J., *Biochim. Biophys. Acta*, **258**, 554-561 (1972).
19. Glazer, A.N., and Smith, E.L., *J. Biol. Chem.*, **236**, 2948-2951 (1961).
20. Pace, C. N., *et al.*, *Protein Sci.*, **4**, 2411-2423 (1995).
21. Arnon, R., *Meth. Enzymol.*, **19**, 226-244 (1970).

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