

## Product Information

# Collagenase from *Clostridium histolyticum*

For General Use

**C0130**

Storage Temperature: 2-8 °C

CAS RN 9001-12-1

EC 3.4.24.3

Synonym: Clostridiopeptidase A

## Product Description

Collagenase from *Clostridium histolyticum* generally refers to a mixture of enzyme activities, mostly various enzymes that hydrolyze collagen, rather than a single enzyme. Six distinct collagenases, labeled  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$  and  $\zeta$ , have been identified from *C. histolyticum* culture filtrate. Within the  $\alpha$  and  $\gamma$  species, two subspecies have been identified ( $\alpha_1$ ,  $\alpha_2$ ;  $\gamma_1$ ,  $\gamma_2$ ).<sup>1-3</sup> These species of individual collagenases have been classified as follows, based on their relative enzymatic activities on native collagen and the synthetic peptide.

*N*-(3-(2-furyl) acryloyl)-Leu-Gly-Pro-Ala (FALGPA)<sup>4</sup>:

- Class I:  $\alpha$ ,  $\beta$ ,  $\gamma$  = high collagenase activity, moderate FALGPA activity
- Class II:  $\delta$ ,  $\epsilon$ ,  $\zeta$  = moderate collagenase activity, high FALGPA activity

Other enzymatic activities have been detected in collagenases isolated from *C. histolyticum*, including elastase and caseinase activities.<sup>1</sup>

Collagenase recognizes the sequence -R-Pro- $\uparrow$ -X-Gly- Pro-R- where X is most often a neutral amino acid.<sup>5</sup> Both zinc ( $\text{Zn}^{2+}$ ) and calcium ( $\text{Ca}^{2+}$ ) are essential metal cofactors for collagenase activity.<sup>3</sup>

Collagens, in their various types, are the natural substrates for collagenase. In addition to FALGPA, many synthetic peptides have been prepared to serve as collagenase substrates, such as:

- *N*-CBZ-Gly-Pro-Gly-Gly-Pro-Ala<sup>7</sup> ( $K_M = 0.71 \text{ mM}$ )<sup>6</sup>
- *N*-CBZ-Gly-Pro-Leu-Gly-Pro<sup>8</sup>

- *N*-2,4-Dinitrophenyl-Pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg<sup>9</sup>
- 4-Phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg<sup>10</sup>

In addition, *N*-Succinyl-Gly-Pro-Leu-Gly-Pro 7-amido-4-methylcoumarin is listed as a substrate for "collagenase-like peptidase".<sup>11</sup> *N*-(2,4-Dinitrophenyl)-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg amide is listed as a substrate for "vertebrate collagenase".<sup>12</sup>

Inhibitors (selected):<sup>6,13</sup>

- Ethylene glycol-bis ( $\beta$ -aminoethyl ether)-*N*, *N*, *N'*, *N'*-tetraacetic acid (EGTA)<sup>13</sup>
- 2-Mercaptoethanol
- Glutathione (reduced)
- Thioglycolic acid sodium salt
- 2,2'-Dipyridyl
- 8-Hydroxyquinoline

Molecular mass:<sup>14</sup> 68,000–125,000 Da  
pH optimum:<sup>6</sup> 6.3–8.8

For use in tissue dissociation, an important factor to consider is the relative ratio of collagenase activity to protease activity. Release of cells from tissue is more effective when both the collagenase and neutral protease activities are present, as either enzyme alone is less effective at cell release.<sup>15</sup>

Collagenase may be used for the disaggregation of human tumor, mouse kidney, human adult and fetal brain, lung, and many other tissues, particularly epithelium. It is also effective in liver and kidney perfusion studies, digestion of pancreas, isolation of nonparenchymal rat liver cells, and hepatocyte preparations.<sup>16-20</sup>

This collagenase product undergoes several activity tests:

- Collagenase: separate tests with bovine achilles tendon and with FALGPA as substrates
- Neutral protease: measured as caseinase
- Clostripain: measured as BAEE after reduction with DTT

This product roughly corresponds to the first 40% ammonium sulfate fraction of Mandl.<sup>21</sup>

### Unit Definitions

One Collagen Digestion Unit (CDU) liberates peptides from bovine achilles tendon equivalent in ninhydrin color to 1.0  $\mu$ mole of leucine in 5 hours, at pH 7.4 at 37 °C, in the presence of calcium ions.

One FALGPA Hydrolysis Unit hydrolyzes 1.0  $\mu$ mole of furylacryloyl-Leu-Gly-Pro-Ala per minute, at 25 °C pH 7.5, in the presence of calcium ions.

One Neutral Protease Unit hydrolyzes casein to produce color equivalent to 1.0  $\mu$ mole tyrosine per 5 hours at pH 7.5 at 37 °C.

One Clostripain Unit hydrolyzes 1.0  $\mu$ mole of BAEE per minute at pH 7.6 at 25 °C in the presence of DTT.

### Storage/Stability

Store the product at 2-8 °C. Solutions of crude collagenase, at 10 mg/mL, are stable if frozen quickly in aliquots and kept frozen at -20 °C. One reference has cited preparation of stock solutions of this product in sterile PBS, containing  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , with 20% calf serum, at 2 mg/mL, and freezing of this stock solution at -20 °C in aliquots (for example, 12 mL), with no more than one freeze-thaw cycle.<sup>22</sup> Repeated freeze-thaw cycles are not recommended. In aqueous solutions, collagenase loses measurable activity in 3 hours at 4 °C. At pH 7.0 in the presence of 1 mM  $\text{Ca}^{2+}$ , there is no loss of activity in 1 hour at 40 °C, 50% loss in 10 minutes at 48 °C, and 100% loss in 5 minutes at 60 °C.<sup>23</sup> The optimal calcium concentration for tissue dissociation is 5 mM.

### 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.

### References

1. Bond, M.D., and Van Wart, H.E., *Biochemistry*, 23(13), 3077-3085 (1984).
2. Bond, M.D., and Van Wart, H.E., *Biochemistry*, 23(13), 3085-3091 (1984).
3. Bond, M.D., and Van Wart, H.E., *Biochemistry*, 23(13), 3092-3099 (1984).
4. Van Wart, H.E., and Steinbrink, D.R., *Anal. Biochem.*, 113(2), 356-365 (1981).
5. *Extracellular Matrix: A Practical Approach* (M.A. Haralson and J.R. Hassell, eds.), Oxford University Press (Oxford, UK), p. 31 (1995).
6. *Enzyme Handbook*, D. Schomberg and M. Salzmann, Editors, Springer-Verlag (Berlin / Heidelberg, Germany), 1991.
7. Grassmann, W., and Nordwig, A., *Hoppe-Seyler's Z. Physiol. Chem.*, 322, 267 (1960).
8. Morita, T., et al., *J. Biochem*, 82(5), 1495-1498 (1977).
9. Gray, R.D., and Saneii, H.H., *Anal. Biochem.*, 120(2), 339-346 (1982).
10. Wuensch, E., and Heidrich, H.G., *Hoppe-Seyler's Z. Physiol. Chem.*, 333, 149-151 (1963).
11. Kojima, K., et al., *Anal. Biochem.*, 100(1), 43-50 (1979).
12. Darlak, K., et al., *J. Biol. Chem.*, 265(9), 5199-5205 (1990).
13. Seglen, P.O., *Meth. Cell Biol.*, 13, 29-83 (1976).
14. *Enzyme Nomenclature 1992* (E.C. Webb, ed.), Academic Press (San Diego, CA), 409 pp. (1992).
15. Briete, A.G., et al., *Transplant Proc.*, 42(6), 2052- 2054 (2010).
16. *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications*, 6th ed. (R.I. Freshney, ed.), John Wiley & Sons (New York, NY), Chapter 11, pp. 163-186 (1994).
17. Kilberg, M.S., *Methods Enzymol.*, 173, 564-575 (1989).
18. Brinckerhoff, C.E., *Methods Enzymol.*, 190, 175- 188 (1990).
19. Chew, C.S., *Methods Enzymol.*, 191, 640-661 (1990).
20. Berglinde, T., *Methods Enzymol.*, 192, 93-107 (1990).
21. Mandl, I., et al., *J. Clin. Invest.*, 32(12), 1323-1329 (1953).
22. Freytes, D.O., et al., in *Cardiac Tissue Engineering: Methods and Protocols* (M. Radisic and L.D. Black III, eds.), *Methods Mol. Biol.*, 1181, 69-81 (2014).
23. Appel, W., in *Methods of Enzymatic Analysis*, 2nd ed. (H. Bergmeyer, ed.), Verlag Chemie Weinheim / Academic Press, 1058-1063 (1974).

## Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

## Technical Assistance

Visit the tech service page at [SigmaAldrich.com/techservice](https://SigmaAldrich.com/techservice).

## Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at [SigmaAldrich.com/terms](https://SigmaAldrich.com/terms).

## Contact Information

For the location of the office nearest you, go to [SigmaAldrich.com/offices](https://SigmaAldrich.com/offices)

The life science business of Merck operates as MilliporeSigma in the U.S. and Canada.

Merck and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.