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Product Information

Collagenase from *Clostridium histolyticum*

high-purity

Catalog Number **C0773**
Storage Temperature –20 °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 α, β, γ, δ, ε, and ζ, have been identified from *C. histolyticum* culture filtrate. Within the α and γ species, two subspecies have been identified (α_1, α_2; γ_1, γ_2).[1-3] 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)[4]:

- Class I: α, β, γ = high collagenase activity, moderate FALGPA activity
- Class II: δ, ε, ζ = moderate collagenase activity, high FALGPA activity

Historically, other enzymatic activities have also been detected in collagenases isolated from *C. histolyticum*, including elastase and caseinase activities.[1] Anion exchange chromatography has been performed on *C. histolyticum* filtrate to obtain further purified collagenase activities, away from other enzymatic activities.[5,6]

Collagenase recognizes the sequence -R-Pro-↑-X-Gly-Pro-R- where X is most often a neutral amino acid.[7] Both zinc (Zn^{2+}) and calcium (Ca^{2+}) are essential metal cofactors for collagenase activity.[3]

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[9] (K_M = 0.71 mM)[8]
- *N*-CBZ-Gly-Pro-Leu-Gly-Pro[10]
- *N*-2,4-Dinitrophenyl-Pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg[11]
- 4-Phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg[12]

In addition, *N*-Succinyl-Gly-Pro-Leu-Gly-Pro 7-amido-4-methylcoumarin is listed as a substrate for "collagenase-like peptidase".[13]

N-(2,4-Dinitrophenyl)-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg amide is listed as a substrate for "vertebrate collagenase".[14]

Inhibitors (selected):[8,15]

- Ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA)[15]
- 2-Mercaptoethanol
- Glutathione (reduced)
- Thioglycolic acid sodium salt
- 2,2′-Dipyridyl
- 8-Hydroxyquinoline

Molecular mass:[16] 68,000–125,000 Da
pH optimum:[6] 6.3–8.8

For use of collagenase products in tissue dissociation, an important factor is the relative ratio of collagenase activity to protease activity. Release of cells from tissue is more effective when both collagenase and neutral protease activities are present, as either enzyme alone is less effective at cell release.[17] This collagenase product has low-neutral protease and low-clostripain activities. Thus this particular collagenase product would require supplemental addition of proteases, for tissue dissociation work.

This product has been used in several applications that specifically focus on collagen degradation, such as:

- Magnetic resonance microscopy of bovine patellar articular cartilage[18]
- Structural studies of the bovine vitreous body[19]
- Micro-computed tomography enhanced by phosphotungstic acid[20]

This collagenase product is a high-purity collagenase that has been purified by chromatography. This product has very high collagen digestion/collagenase activity compared to FALGPA activity. It 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

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

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

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

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

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.

Storage/Stability
Store the product at –20 °C.

Solutions of this collagenase product at 1,000 units/mL, in 100 mM Trizma®-HCl, pH 7.4, 1 mM $CaCl_2$, and 0.1% Triton™ X-100 aqueous buffer, are stable if frozen quickly in 50–100 μL aliquots and kept frozen at –20 °C.[21] 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 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.[22] The optimal calcium concentration for tissue dissociation is 5 mM.

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