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Product Information

Collagenase from *Clostridium histolyticum*

crude, for adipocyte and hepatocyte isolation

Catalog Number **C2139**
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

Other enzymatic activities have been detected in collagenases isolated from *C. histolyticum*, including elastase and caseinase activities.[1]

Collagenase recognizes the sequence -R-Pro-↑-X-Gly-Pro-R- where X is most often a neutral amino acid.[5] 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[7] (K_M = 0.71 mM)[6]
- *N*-CBZ-Gly-Pro-Leu-Gly-Pro[8]
- *N*-2,4-Dinitrophenyl-Pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg[9]
- 4-Phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg[10]

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

Inhibitors (selected):[6,13]

- Ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA)[13]
- 2-Mercaptoethanol
- Glutathione (reduced)
- Thioglycolic acid sodium salt
- 2,2′-Dipyridyl
- 8-hydroxyquinoline

Molecular mass:[14] 68,000–125,000 Da
pH optimum:[6] 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.[15]

This product is suitable for the release of viable hepatocytes from rat liver by the method of Seglen.[13] It is also suitable for the isolation of fat cells from rat adipose tissue by the method of Rodbell.[16]

This product may also 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.[17-21]

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.[22]

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 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.

Stock solutions of this product can be prepared at 100 mg/mL in DMEM/F12, and stored at –20 °C in frozen aliquots (e.g., 200 μL).[23] Solutions of crude collagenase are stable if frozen quickly in aliquots (at 10 mg/mL) and kept frozen at –20 °C. Repeated freeze-thaw cycles are not recommended. In aqueous solutions, bacterial 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.[24] The optimal calcium concentration for tissue dissociation is 5 mM. The product retains 100% activity over 7 hours when held on ice.[20]

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