Product Information Sheet

Collagenase from Clostridium histolyticum

Sterile filtered

C2014

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). 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).

- 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.

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

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 (K_m = 0.71 mM)
- *N*-CBZ-Gly-Pro-Leu-Gly-Pro
- *N*-2,4-Dinitrophenyl-Pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg
- 4-Phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg

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

Inhibitors (selected):

- Ethylene glycol-bis (β aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA)
- 2-mercaptoethanol
- Glutathione (reduced)
- Thioglycolic acid sodium salt
- 2,2′-dipyridyl
- 8 hydroxyquinoline

Molecular mass: 68,000-125,000 Da

pH optimum: 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.

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 preparation.

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.

This product has been prepared from Catalogue No. C9263, by sterile filtration.

Unit Definitions

One Collagen Digestion Unit (CDU) liberates peptides from bovine achilles tendon equivalent in ninhydrin color to 1.0 µ mole 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.

Sterility

The lot passes the requirements of the test for sterility (USP XXII, pp 1483-1488, 1990).

Storage/Stability

Store the product at –20 °C. 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, 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. The optimal calcium concentration for tissue dissociation is 5 mM.

Precautions and Disclaimer

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.

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C2014pis Rev 08/22