Collagenase from *Clostridium histolyticum*
crude, for adipocyte isolation

Catalog Number C6885
Storage Temperature –20 °C

CAS RN 9001-12-1
EC 3.4.24.3
Synonym: Clostridiopeptidase A

**Product Description**
Although the term collagenase implies there is a single enzyme produced by *C. histolyticum*, this is not the case. Crude collagenase preparations are mixtures of enzyme activities (mostly proteases) secreted by *C. histolyticum*. All may contain 10 to 18 components (by electrophoresis), only 8 of which have been identified. The collagenase products differ by the amount of the components absolute and relative to each other.

Collagenase recognizes the sequence -R-Pro↑-X-Gly-Pro-R- where X is most often a neutral amino acid. It is activated by four gram atoms of calcium (Ca^{2+}) per mole of enzyme.

Substrates:
The various types of collagen are the natural substrates for collagenase. Many synthetic peptides have been prepared to serve as collagenase substrates; they include: N-CBZ-gly-pro-gly-gly-pro-alal\(^3\) (K\(m\) = 0.71 mM);\(^2\) N-CBZ-gly-pro-leu-gly-pro;\(^4\) N-2,4-Dinitrophenyl-pro-gln-gly-leu-ala-gly-gln-D-arg;\(^5\) N-(3-(2-furyl)acyloyl)-leu-gly-pro-ala (FALGPA);\(^6\) 4-Phenylazobenzoylcarbonyl-pro-leu-gly-pro-D-arg.\(^7\) In addition N-Succinyl-gly-pro-leu-gly-pro 7-amido-4-methylcoumarin is listed as a substrate for "collagenase-like peptidase" and N-(2,4-Dinitrophenyl)-pro-leu-gly-leu-trp-ala-D-arg amide is listed as a substrate for "vertebrate collagenase".\(^8\)

Inhibitors:
Inhibitors of collagenase include ethylene glycol-bis(β-aminooethyl ether)-N,N,N',N'-tetraacetic acid (EGTA);\(^9\) 2-mercaptoethanol; glutathione, reduced; thioglycolic acid, sodium; 2,2'-dipyridyl; and 8-hydroxyquinoline.\(^1\)

Molecular mass: \(68,000\) to \(125,000\)

pH optimum: \(6.3-8.8\)

This crude collagenase preparation contains two specific collagenases (measured as FALGPA units/mg), clostripain (measured as BAEE after reduction with DTT), and a neutral protease (measured as caseinase). It is equivalent to the first 40% ammonium sulfate fraction of Mandl.\(^1\)

In the crude collagenase preparation, the clostripain is mostly inactive, oxidized. It contains no carbohydrate and no salts are present.

An important feature for use in tissue dissociation is the ratio of collagenase to protease. Effective release of cells from tissue depends on the action of both the two collagenase enzymes and the neutral protease, for neither one alone is not very effective.\(^1\)

This product is suitable for the isolation of fat cells from rat adipose tissue by the method of Rodbell.\(^1\) Fat cells can be screened for metabolic integrity by measuring glucose oxidation rates with and without insulin addition.

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 preparation.\(^1\)
Unit definitions:
One Collagen Digestion Unit (CDU) liberates peptides from collagen equivalent in ninhydrin color to 1.0 μmole of leucine in 5 hr 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 min 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 hr at pH 7.5 at 37 °C.

One Clostripain Unit hydrolyzes 1.0 μmole of BAEE per min 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. There is no loss in FALGPA or protease activity in 30 days at 37 °C, 50 °C, and −20 °C. Solutions of crude collagenase are stable if frozen quickly in aliquots (at 10 mg/mL) and kept frozen at −20 °C. Freeze-thaw cycles will damage the enzyme solution. In aqueous solutions bacterial collagenase loses measurable activity in 3 hr. at 4 °C. At pH 7.0 in the presence of 1 mM Ca²⁺ there is no loss of activity in 1 hr. at 40 °C, 50 % loss in 10 min at 48°C and 100% loss in 5 min. at 60 °C. The optimal calcium concentration for tissue dissociation is 5 mM. The product retains 100% activity over 7 hours when held on ice.

References
13. Personal, Dr. James Gill, Sigma Chemical Co.
20. Sigma data.