COLLAGENASE, CRUDE, General Use
from Clostridium histolyticum
Sigma Prod. No. C0130

CAS: 9001-12-1
ENZYME COMMISSION NUMBER: 3.4.24.3
SYNONYMS: Clostridiopeptidase A

PHYSICAL DESCRIPTION:

Appearance: Tan to brown powder
Molecular weight: 68,000 to 125,000\(^1\)
Isoelectric point: Not determined
pH optimum: 6.3-8.8\(^2\). The enzyme is assayed by Sigma at pH 7.4.
Salts present: None
Specificity: Collagenase recognizes the sequence -R-Pro-\(\beta\)-X-Gly-Pro-R- where X is most often a neutral amino acid.\(^3\)

COMPOSITION:

Crude collagenases are mixtures of enzymes (mostly proteases) secreted by Clostridium histolyticum. This preparation contains collagenase, non-specific proteases and clostripain. It contains no carbohydrate.\(^2\)

ACTIVATORS:

Collagenase is activated by four gram atom calcium (Ca\(^{2+}\)) per mole enzyme.\(^2\)

INHIBITORS:

Inhibitors of collagenase include ethylene glycol-bis(\(\beta\)-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)\(^4\); \(\beta\)-mercaptoethanol; glutathione, reduced; thioglycolic acid, sodium; 2,2'-dipyridyl; 8-hydroxyquinoline.\(^2\)

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-pro-ala\(^5\) (\(K_m = 0.71 \text{ mM}\)); N-CBZ-gly-pro-leu-gly-pro\(^6\); N-2,4-Dinitrophenyl-pro-gln-gly-ile-ala-gly-gln-D-arg\(^7\); N-(3-(2-furyl)acryloyl)-leu-gly-pro-ala (FALGPA)\(^8\); 4-Phenylazobenzyloxycarbonyl-pro-leu-gly-pro-D-arg\(^9\). In addition N-Succinyl-gly-pro-leu-gly-pro 7-amido-4-methylcoumarin is listed as a substrate for "collagenase-like peptidase"\(^10\) and N-(2,4-Dinitrophenyl)-pro-leu-gly-leu-trp-ala-D-arg amide is listed as a substrate for "vertebrate collagenase".\(^11\)
APPLICATIOnS:

This product is suitable 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.\textsuperscript{15-19}

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

The component enzymes in crude are two specific collagenases (measured as FALGA units/mg), clostripain (measured as BAEE after reduction with DTT) and a neutral protease (measured as caseinase). In the crude collagenase, the clostripain is mostly inactive, oxidized. 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 either alone is not very effective.\textsuperscript{12}

METHOD OF PREPARATION:

Crude collagenase is equivalent to the first 40\% ammonium sulfate fraction of Mandl.\textsuperscript{13}

STABILITY / STORAGE AS SUPPLIED:

This product is stable for at least one year when stored at -20°C. There is no loss in FALGPA or protease activity in 30 days at 37°C, 50°C and -20°C.\textsuperscript{20}

SOLUTION / SOLUTION STABILITY:

Usually, solutions are prepared at 1-2 mg/ml in TESCA buffer (50 mM TES, 0.36 mM Calcium chloride, pH 7.4 at 37°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 Ca\textsuperscript{2+} 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.\textsuperscript{14} The optimum calcium concentration for tissue dissociation is 5 mM. The product retains 100\% activity over 7 hours when held on ice.\textsuperscript{20}
COLLAGENASE, CRUDE, General Use
from *Clostridium histolyticum*
Sigma Prod. No. C0130

UNIT DEFINITIONS:

One Collagen Digestion Unit 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.

REFERENCES:

12. Personal, Dr. James Gill, Sigma Chemical Co.
20. Sigma data
Sigma warrants that its products conform to the information contained in this and other Sigma\Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.