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COLLAGENASE, CRUDE,
For adipocyte and hepatocyte isolation
from *Clostridium histolyticum*
Sigma Prod. No. C2139

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 125,000¹

Isoelectric point: Not determined

pH optimum: 6.3-8.8². The enzyme is assayed by Sigma at pH 7.4.

Salts present: None

Specificity: Collagenase recognizes the sequence -R-Pro-1-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 atoms of calcium (Ca²⁺) per mole of enzyme.²

INHIBITORS:

Inhibitors of collagenase include ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)⁴; β-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-gly-pro-ala⁵ (K_m = 0.71 mM²); N-CBZ-gly-pro-leu-gly-pro⁶; N-2,4-Dinitrophenyl-pro-gln-gly-ile-ala-gly-gln-D-arg⁷; N-(3-(2-furyl)acryloyl)-leu-gly-pro-ala (FALGPA)⁸; 4-Phenylazobenzoyloxycarbonyl-pro-leu-gly-pro-D-arg⁹. 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

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APPLICATIONS:

This product is suitable for the release of viable hepatocytes from rat liver by the method of Seglen.⁴ It is also suitable for the isolation of fat cells from rat adipose tissue by the method of Rodbell.¹² Fat cells are then 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.¹³⁻¹⁷

Although the term collagenase implies that there is a single enzyme produced by *Cl. histolyticum*, this is not the case. Crude collagenases are mixtures of enzyme activities (mostly proteases) secreted by *Cl. 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 FALGPA 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.¹⁸

METHOD OF PREPARATION:

Crude collagenase is equivalent to the first 40% ammonium sulfate fraction of Mandl.¹⁹

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.²⁰

SOLUTION / SOLUTION STABILITY:

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.²¹ The optimum 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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UNIT DEFINITIONS:

One Collagen Digestion Unit liberates peptides from collagen equivalent in ninhydrin color to 1.0 μ mole 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 μ mole 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 μ mole tyrosine per 5 hr at pH 7.5 at 37°C.

One Clostripain Unit hydrolyzes 1.0 μ mole of BAEE per min at pH 7.6 at 25°C in the presence of DTT.

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