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Product Information

COLLAGENASE, HIGH-PURITY, from *Clostridium histolyticum*

Sigma Prod. No. **C0773**

CAS NUMBER: 9001-12-1

ENZYME COMMISSION NUMBER: 3.4.24.3

SYNONYMS: Clostridiopeptidase A

PHYSICAL DESCRIPTION:

Appearance: White to tan 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: Calcium chloride

Specificity: Collagenase recognizes the sequence -R-Pro-8-X-Gly-Pro-R- where X is most often a neutral amino acid.³

COMPOSITION:

This product contains approximately 95% protein (Biuret). The balance is primarily calcium chloride. A typical SDS electrophoretic analysis gives 40% at 107 kDa, 28% at 81 kDa, 7% at 100 kDa, 5% at 77 kDa and 5% at 63 kDa plus several additional trace bands.

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

APPLICATIONS:

This collagenase was used by Makita¹² in the preparation of arterial tissue for the study of Advanced Glycosylation End Products (AGE). Collagenase was added at 1 mg to 100 mg tissue and the mixture incubated for 48 hrs at 37°C with gentle shaking.

This product, when used with added proteases, 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 hepatocytes.¹³⁻¹⁷

METHOD OF PREPARATION:

This product is prepared from *Clostridium histolyticum* using ion exchange chromatography. It contains no detectable protease, tryptic and clostripain activity.¹⁸

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.¹⁸

SOLUTION / SOLUTION STABILITY:

A stock solution may be prepared by dissolving 0.05-0.1 mg collagenase in one ml of 50 mM TES buffer containing 0.36 mM calcium chloride (TESCA), pH 7.4 at 37°C.

Solutions of collagenase are stable if frozen quickly in aliquots 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.¹⁹ The optimum calcium concentration for tissue dissociation is 5 mM. The product retains 100% FALGPA activity over 7 hours when held on ice.¹⁸

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.

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