Product Information

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Collagenase
from *Clostridium histolyticum*
Type IA, crude, suitable for general use

Catalog Number **C9891**
Storage Temperature –20 °C

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

Product Description
"Crude" collagenase refers to the material purified from the fermentation of *Clostridium histolyticum* bacteria. It is actually a mixture of several different enzymes including collagenase, which act together to break down tissue. This preparation contains collagenase, non-specific proteases, clostripain, neutral protease, and aminopeptidase activities. Crude collagenase is equivalent to the first 40% ammonium sulfate fraction prepared by Mandl.[1]

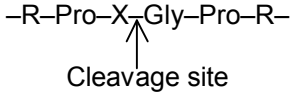
Molecular mass (SDS-PAGE):[2,3] 68–130 kDa
As many as seven collagenase proteins can be present, some of these are C-truncated forms of the Type I and Type II collagenases (sometimes called collagenases A and B) that are expressed from two genes, *colG* and *colH*.[3]

Molecular mass (sequence): The *colG* and *colH* genes have been isolated from *C. histolyticum* JCM 1403 (ATCC 19401).[3,4] The *colG* gene encodes a mature 113,897 Da Type I collagenase and several C-truncated forms. The *colH* gene encodes the mature 111,963 Dal Type II collagenase and one C-truncated form.

Optimal pH:[5] 6.3–8.8 The enzyme is assayed at pH 7.4

Isoelectric Point (pI): Six collagenases purified from crude collagenase had isoelectric points in the range of 5.35–6.20.[6]

Specificity: collagenase recognizes the following peptide sequence where X is most often a neutral amino acid:[7]



Substrates: In addition to the various natural collagen substrates, many synthetic substrates have been prepared:[8–14]

Z-Gly-Pro-Gly-Gly-Pro-Ala (Catalog Numbers 27673 and 27670; K_M = 0.71 mM)[2]
Z-Gly-Pro-Leu-Gly-Pro
N-2,4-Dinitrophenyl-Pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg
N-(3-(2-furyl)acryloyl)-Leu-Gly-Pro-Ala (FALGPA, Catalog Number F5135)
4-Phenylazobenzoxycarbonyl-Pro-Leu-Gly-Pro-D-Arg
N-succinyl-Gly-Pro-Leu-Gly-Pro 7-amido-4-methyl-coumarin (substrate for "collagenase-like peptidase")
N-(2,4-Dinitrophenyl)-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg amide (substrate for "vertebrate collagenase")

Activators/Cofactors: Collagenase activity is stabilized by 0.1 mole calcium ions (Ca^{2+}) per mole of enzyme.[4] Calcium ions also facilitate binding to the collagen molecule.[18] Zinc ions (Zn^{2+}) are required for activity, but are tightly bound to the collagenase during purification.[19] Additional Zn^{2+} should not be necessary as long as no chelator is added during digestion.

Inhibitors:
Ethylene glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetra-acetic acid (EGTA, Catalog Number E0396)[15]
Ethylenediaminetetraacetic acid (EDTA, Catalog Number E6758)[16]
β-Thujaplicin (Hinokitiol)[17]
Cysteine[16]
2-mercaptoethanol (Catalog Number M7522)[16]
Glutathione (reduced)
Thioglycolic acid, sodium
8-Hydroxyquinoline-5-sulfonate[16]

This product is supplied as a lyophilized powder. Traces of calcium chloride and sodium bicarbonate may be present.

Collagenase activity: measured with two substrates; the FALGPA assay measures digestion of a short artificial peptide[11] and the Collagen Digestion Unit (CDU) assay measures digestion of native collagen. Proteolytic activities are assayed with a substrate prepared from casein. Clostripain activity requires a reduced environment so this enzyme has little activity in this preparation.

Specific activity: ≥125 CDU/mg solid

Unit Definition: One Collagen Digestion Unit (CDU) liberates peptides from collagen equivalent in ninhydrin color to 1.0 μmole of L-leucine in 5 hours at pH 7.4 at 37 °C in the presence of calcium ions.
Note: Mandl units have the same description as Sigma Collagenase Digestion Units. The conversion factor for Mandl units/Wuensch units to Sigma units is 1000-2000 to 1.

Specific activity: 0.5–5.0 FALGPA units/mg solid

Unit Definition: 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.

Neutral Protease (caseinase) activity:
≤350 units/mg solid

Unit Definition: 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.

Clostripain activity: ≤4 units/mg solid

Unit Definition: One Clostripain Unit hydrolyzes 1.0 μmole of BAEE per minute 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 Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Reconstitution Buffers:
For enzyme assays: 50 mM TES (Catalog Number T1375) with 0.36 mM $CaCl_2$ (Catalog Number C3881), pH 7.4, at 37 °C.

For tissue dissociations: Krebs-Ringer Bicarbonate Buffer (Catalog Number K4002) with added calcium (0.3–0.5 mM) and BSA (1%).

For tissue culture applications: calcium-free solutions such as Hank's Balanced Salts (Catalog Number H2387) or Earle's Balanced Salt Solution (Catalog Number E6267).

To sterile filter solutions of crude collagenase, first centrifuge the solution or filter through a 0.8 μm filter to remove insolubles. This pretreatment will reduce the clogging of the 0.2 μm filter during sterile filtration.

Storage/Stability
This product retains activity for at least one year when stored at –20 °C.

Solutions (10 mg of protein/ml) at neutral pH and with adequate calcium ion concentration (0.3–0.5 mM) will retain activity for over 6 hours on ice.[20] Solutions at –20 °C are stable for several months if frozen quickly in aliquots (10 mg/ml).[4] Freeze-thaw cycles will reduce enzyme activities.

Procedure
This product is suitable for the disaggregation of mouse kidney, human adult and fetal brain, lung, tumor, and many other tissues, particularly epithelium. It is also effective in liver and kidney perfusion studies, digestion of pancreas, isolation of non-parenchymal rat liver cells, and hepatocyte preparation.[21-29]

The release of cells from tissue depends on the action of both collagenase enzymes and the neutral protease. An important feature for tissue dissociation is the ratio of collagenase to protease activity. Protein concentrations typically vary from 0.1–5 mg/ml and digestion time should be monitored using very gentle agitation to check for tissue dissociation. Collagenase treatment can cause some cells to die. A satisfactory degree of cell dissociation without too much cell death typically can be achieved from 15 minutes to several hours, but can fall outside this range if the protein concentration is not in the typical range.

References

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