Version 3. Dec 23rd 2011

TECHNICAL DATASHEET

COLLAGENASE G/H PURE70

Name of the enzyme: **COLLAGENASE G/H**

Organism of origin: *Clostridium histolyticum*

Recombinant production in: *Escherichia coli* Rosetta (DE3) pLysS, the enzyme contains a 6-histidine tag in its C-terminus end.

CAS: 9001-12-1
ENZYME COMMISSION NUMBER: 3.4.24.3
SYNONYMS: ColH/G, Microbial collagenase

PHYSICAL DESCRIPTION:
Appearance: white powder
Form: Lyophilized powder
Quality: Purified by salt precipitation
Storage Temperature: Room temperature
Long Term Storage Temperature: -20ºC/-80ºC

SPECIFICITY: ColG degrades the helical regions in native collagen preferentially at the Y-Gly bond in the sequence Pro-Y-Gly-Pro-, where Y is most frequently a neutral amino acid[1]. Collagenase G preferentially acts on intact collagen fibers. ColH degrades single strands of collagen rather than triple helical regions in native collagen. This enzyme preferentially cleaves at the Y-Gly bond in the sequence Pro-Y-Gly-Pro-, where Y is most frequently a neutral amino acid[2]

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PROPERTIES:

Molecular weight	**112,98-114,8**
Optimum pH:	**8,0**
Isoelectric point:	**5,64**
Optimum Tª (ºC)	**37**

COMPOSITION:

Identifiers	Number	Name
CAS number	9001-12-1	ColH/G
CAS number	7647-14-5	$(NH_4)_2SO_4$
CAS number	77-86-1	$(HOCH_2)_3CNH_2$
CAS number	10043-52-4	$CaCl_2$
CAS number	7647-01-0	HCl

TECHNICAL DATASHEET

COLLAGENASE G/H PURE70

PURE70 COLLAGENASE G/H is a salt precipitate's purified protein. For this reason, ***PURE70 COLLAGENASE G/H*** is suitable for its application in research and some techniques of biomedicine which not require at high purity degree.

ACTIVATORS:

PURE70 COLLAGENASE G/H is activated by four gram atom calcium (Ca^{2+}) per mole enzyme[3].

INHIBITORS:

PURE70 COLLAGENASE G/H inhibitors include: 1,10-phenanthroline[4], 2-(1-benzyl-3-naphthalen-1-yl-ureido)-3-methyl-butyric acid[5], 2-(1-benzyl-3-naphthalen-1-yl-ureido)-N-hydroxy-3-methyl-butyramide[4], 2-(benzyl[[(2,4-difluorophenyl)amino]carbonyl]amino)-3-methylbutanoic acid[4], 2-mercaptoethanol[6], 2-[benzyl(1-naphthylsulfonyl)amino]propanoic acid[7], 2-[benzyl(5-methylnaphthalene-1-sulfonyl)amino]-3-methylbutanoic acid[4], Linoleic acid[8], Linolenic acid[7] and stearic acid[7].

SUBSTRATES:

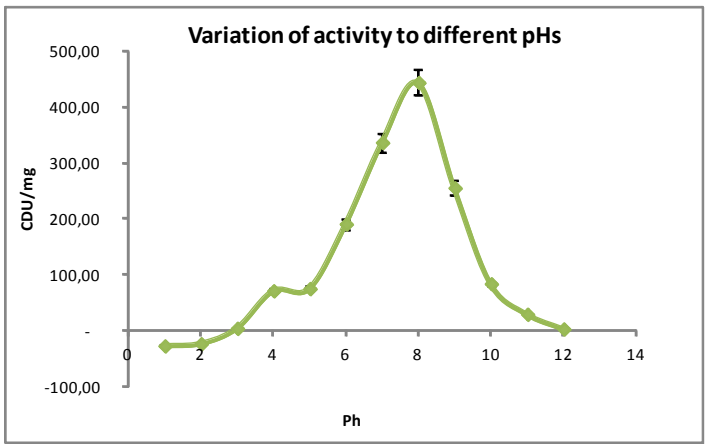
PURE70 COLLAGENASE G/H is a soluble protein in water or aqueous buffers.
Pure70 Collagenase G/H is a metalloproteinase (type II) with capacity for hydrolyzing specifically collagen helix regions at the motif Pro-Y-Gly-Pro characteristic of the collagen proteins. Our recombinant collagenase bears a 6-His tag at the carboxyl end.

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-ala[9] (Km = 0.71 mM[10]); N-CBZ-gly-pro-leu-gly-pro[11]; N-2,4-Dinitrophenyl-pro-gln-gly-ile-ala-gly-gln-D-arg[12]; N-(3-(2-furyl)acryloyl)-leu-gly-pro-ala (FALGPA)[13]; 4-Phenylazobenzyloxycarbonyl-pro-leu-gly-pro-D-arg[14]. In addition N-Succinyl-gly-pro-leu-glypro 7-amido-4-methylcoumarin is listed as a substrate for "collagenase-like peptidase"[15] and N-(2,4-Dinitrophenyl)-proleu-gly-leu-trp-ala-D-arg amide is listed as a substrate for "vertebrate collagenase"[16].

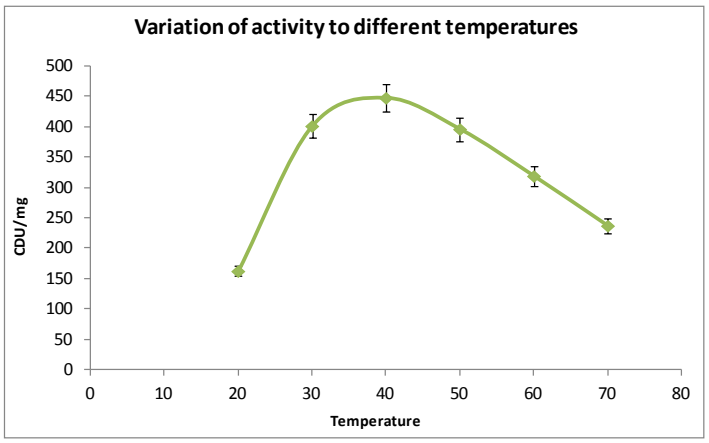
ENZYMATYC ACTIVITY AND CHARACTERIZATION

PURE70 COLLAGENASE G/H has an activity of >1500 CDU/mg, with Tris-HCl buffer (using collagen as substrate at 37 ºC) depending on the pH and temperature range.

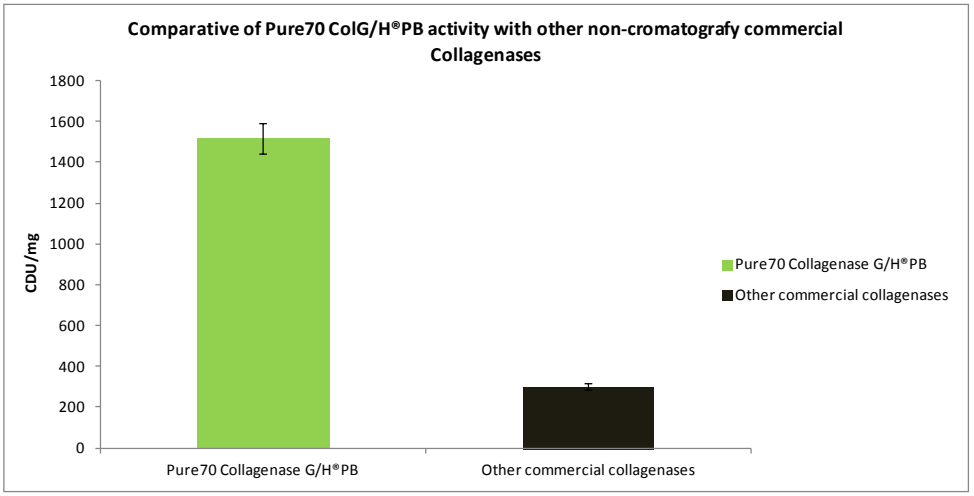
PURE70 COLLAGENASE G/H does not present unspecific activities because these two proteins has been produced by DNA recombinant technology.



The best enzymatic activity is observed at 40ºC where the enzyme shows good stability.



The activity comparative analysis with other commercials Collagenase shows ***PURE100*** and ***PURE70 Collagenase G/H*** has around 3-6 times more activity than the best collagenase in the market (test in vitro at pH 8.0; 37 ºC).

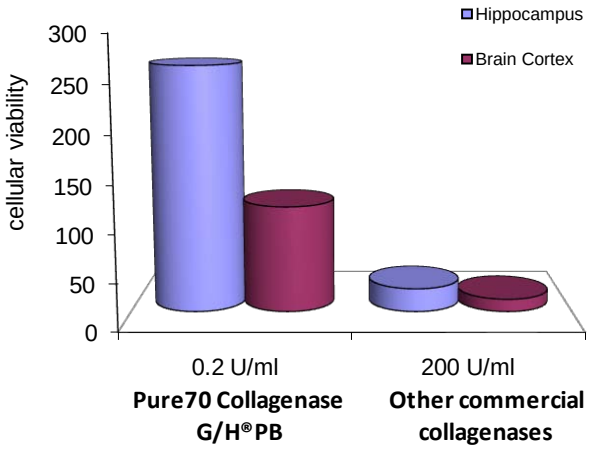


APPLICATIONS

PURE70 COLLAGENASE G/H is especially indicated for the isolation of primary cells from brain cortex, hippocampus, cerebellum, liver, pancreas, heart, and stem cells. In addition, it is widely used in established cell lines.

PURE70 COLLAGENASE G/H is required at low concentrations for the isolation of cells from tissues due to its high purity and specificity. With this new tool for cell culture it is possible to significantly increase the yield of tissue dissociation and cell viability.

Studies done by independent scientific groups show a 10 times increase in the cellular viability of hippocampus and brain cortex cultures compared to other conventional enzymes. Furthermore, for this type of tissues ***PURE70 COLLAGENASE G/H*** is required in up to 1.000 times lower amounts to achieve these results.



METHOD OF PREPARATION:

PURE70 Collagenase G/H is provided as a lyophilized powder and is stable at room temperature. For long term storage, we recommend storing the product at -20ºC/-80ºC for enzymatic activity preservation.
We recommend dissolving the enzyme immediately before using it or to store in aliquots at -20ºC for better preservation of the activity. We recommend avoiding multiple freeze-thaw cycles and exposure to frequent temperature changes.

PURE70 Collagenase G/H is provided in four formats, 30 mg, 60 mg, 120 mg and 300 mg. The enzyme is soluble in water and diluted salts solution; depending of the application of the enzyme, it can be dissolved in both.

The re-constitutive buffer of the enzyme is composed by 20 mM Tris-HCl buffer pH 8.0.
We recommend dissolving the enzyme in 1 ml of re-constitutive buffer in order to make an enzymatic stock solution and aliquot for storing at -20ºC/-80ºC.

Stock solution must be diluted in the re-constitutive buffer or can be directly added into the solution where the enzyme is going to be working, in order to achieve the required enzymatic activity.

The working solution must be prepared just prior usage and remains stable if stored at 2-8ºC for 4-5 days or for long-term use if frozen at -20ºC for better preservation of the original activity.

STABILITY/STORAGE AS SUPPLIED

PURE70 Collagenase G/H is provided as a lyophilized powder and is stable at room temperature. For long term storage, we recommend storing the product at -20ºC/-80ºC for enzymatic activity preservation.
This product is stable for at least one year when stored at -20ºC/-80°C.

SOLUTION/SOLUTION STABILITY

Usually, solutions are prepared in Tris-HCl (20 mM, pH 8.0). If the application permits, we recommend adding 0.36 mM NaCl at the working solution for improving the activity of the enzyme.

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

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