



## TECHNICAL DATASHEET

### COLLAGENASE G/H PURE100

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

**Organism of origin:** *Clostridium histolyticum*

**Recombinant production in:** *Escherichia coli* BL21 DE3, 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: Nickel Affinity Chromatography

Storage Temperature: -20°C/-80°C

**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<sup>1</sup>. 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<sup>2</sup>.

#### PROPERTIES:

<b>Molecular weight</b>	<b>112,98-114,8</b>
<b>Optimum pH:</b>	<b>8,0</b>
<b>Isoelectric point:</b>	<b>5,64</b>
<b>Optimum T<sup>a</sup> (°C)</b>	<b>37</b>

#### COMPOSITION:

<b>Identifiers</b>	<b>Number</b>	<b>Name</b>
CAS number	9001-12-1	ColH/G
CAS number	7647-14-5	NaCl
CAS number	77-86-1	(HOCH <sub>2</sub> ) <sub>3</sub> CNH <sub>2</sub>
CAS number	10043-52-4	CaCl <sub>2</sub>

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**PURE100 COLLAGENASE G/H** is an Affinity Chromatographically purified protein, highly pure (95%). For this reason, **PURE100 COLLAGENASE G/H** is suitable for its application in research and biomedicine.

#### ACTIVATORS:

**PURE100 COLLAGENASE G/H** is activated by four gram atom calcium ( $\text{Ca}^{2+}$ ) per mole enzyme<sup>3</sup>.

#### INHIBITORS:

**PURE100 COLLAGENASE G/H** inhibitors include: 1,10-phenanthroline<sup>4</sup>, 2-(1-benzyl-3-naphthalen-1-yl-ureido)-3-methyl-butyrac acid<sup>5</sup>, 2-(1-benzyl-3-naphthalen-1-yl-ureido)-N-hydroxy-3-methyl-butylamide<sup>4</sup>, 2-(benzyl[[[(2,4-difluorophenyl)amino]carbonyl]amino]-3-methylbutanoic acid<sup>4</sup>, 2-mercaptoethanol<sup>6</sup>, 2-[benzyl(1-naphthylsulfonyl)amino]propanoic acid<sup>7</sup>, 2-[benzyl(5-methylnaphthalene-1-sulfonyl)amino]-3-methylbutanoic acid<sup>4</sup>, Linoleic acid<sup>8</sup>, Linolenic acid<sup>7</sup> and stearic acid<sup>7</sup>.

#### SUBSTRATES:

**PURE100 COLLAGENASE G/H** is a soluble protein in water or aqueous buffers.

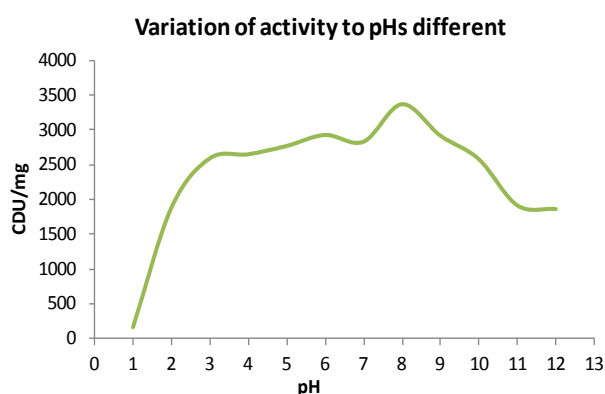
*Pure100 Collagenase G/H* is a metalloproteinase (type II) with capacity for hydrolyzing specific collagen helix regions at the motif Pro-Y-Gly-Pro characteristic of the collagen proteins. Our recombinant Collagenase bears a 6-His tag at the carboxyl end.

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<sup>9</sup> ( $K_m = 0.71 \text{ mM}^{10}$ ); N-CBZ-gly-pro-leu-gly-pro<sup>11</sup>; N-2,4-Dinitrophenyl-pro-gln-gly-ile-ala-gly-gln-D-arg<sup>12</sup>; N-(3-(2-furyl)acryloyl)-leu-gly-pro-ala (FALGPA)<sup>13</sup>; 4-Phenylazobenzoyloxycarbonyl-pro-leu-gly-pro-D-arg<sup>14</sup>. In addition N-Succinyl-gly-pro-leu-glypro 7-amido-4-methylcoumarin is listed as a substrate for "collagenase-like peptidase"<sup>15</sup> and N-(2,4-Dinitrophenyl)-proleu-gly-leu-trp-ala-D-arg amide is listed as a substrate for "vertebrate collagenase"<sup>16</sup>.

#### ENZYMATIC ACTIVITY AND CHARACTERIZATION

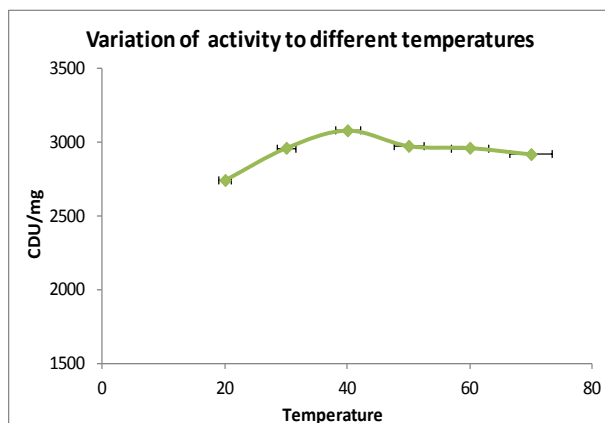
**PURE100 COLLAGENASE G/H** has an activity of >2500 CDU/mg, with Tris-HCl buffer (using collagen as substrate at 37 °C) depending on the pH range.



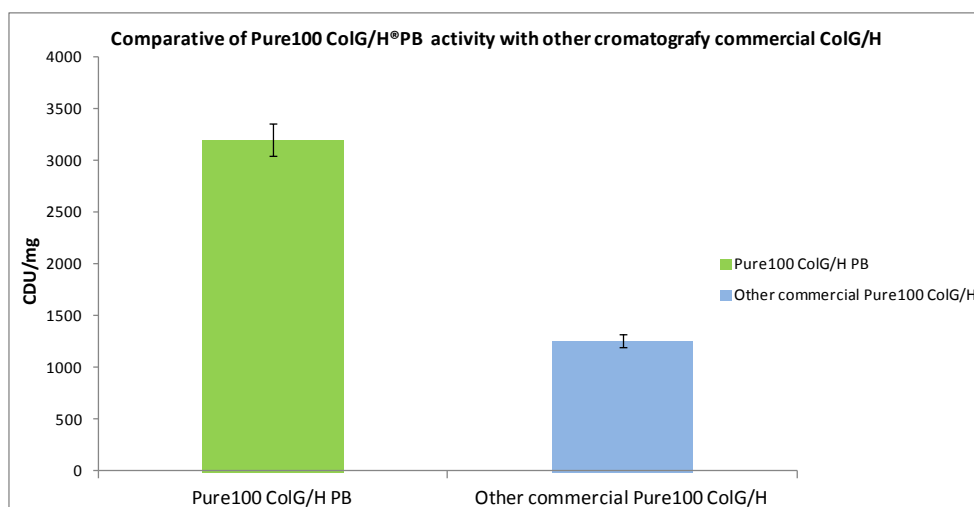
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The best enzymatic activity is observed at 40°C where the enzyme shows good stability.



The activity comparative analysis with other commercial Collagenases shows **PURE100 Collagenase G/H** has around 4-10 times more activity than the best Collagenase in the market (test in vitro at pH 8.0; 37 °C).



The enzyme shows no detectable caseinase activity.

## APPLICATIONS

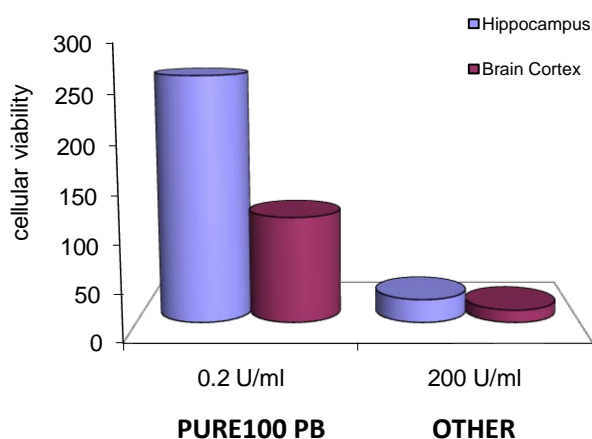
**PURE100 COLLAGENASE G/H** is especially indicated for the isolation of primary cells from brain cortex, hippocampus, cerebellum, liver, pancreas, heart, and stem cells. In addition, it is widely used in established cell lines.

**PURE100 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.

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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 **PURE100 COLLAGENASE G/H** is required in up to 1.000 times lower amounts to achieve these results.



#### METHOD OF PREPARATION:

**PURE100 Collagenase G/H** is provided as a lyophilized powder and remains stable without any significant loss of activity when stored at  $-20^{\circ}\text{C}/-80^{\circ}\text{C}$ . We recommend dissolving the enzyme immediately before using it or to store in aliquots at  $-20^{\circ}\text{C}$  for better preservation of the activity. We recommend avoiding multiple freeze-thaw cycles and exposure to frequent temperature changes.

**PURE100 Collagenase G/H** is provided in two formats, 1.500 U and 7.500 CDU. The enzyme is soluble in water and diluted salts solution; depending of the application of the enzyme 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 (1.500 CDU/ml and 7.500 CDU/ml respectively) and aliquot for storing at  $-20^{\circ}\text{C}/-80^{\circ}\text{C}$ .

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  $2-8^{\circ}\text{C}$  for 4-5 days or for long-term use if frozen at  $-20^{\circ}\text{C}$  for better preservation of the original activity.

#### STABILITY/STORAGE AS SUPPLIED

This product is stable for at least one year when stored at  $-80^{\circ}\text{C}$ .

### SOLUTION/SOLUTION STABILITY

Usually, solutions are prepared in Tris-HCl (20 mM, pH 8.0). If the application permits it, 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  $\mu$ 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  $\mu$ 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  $\mu$ mole tyrosine per 5 hr at pH 7.5 at 37°C”.

“One Clostripain Unit hydrolyzes 1.0  $\mu$ mole of BAEE per min at pH 7.6 at 25°C in the presence of DTT”.

### REFERENCES

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**Li StarFish S.r.l.**  
Via Cavour, 35  
20063 Cernusco S/N (MI)  
telefono 02-92150794  
fax 02-92157285  
info@listarfish.it  
www.listarfish.it