Version 3. Dec 23rd 2011

# TECHNICAL DATASHEET

## PURE100 COLLAGENASE G

**Name of the enzyme**: **COLLAGENASE G**
**Organism of origin**: *Clostridium histolyticum*
**Recombinant production in**: *Escherichia coli* Rosetta (DE3) pLysS (Novagen; Ref: 70956), the enzyme contains a 6-histidine tag in its C-terminus end.

**CAS:** 9001-12-1
**ENZYME COMMISSION NUMBER**: 3.4.24.3
**SYNONYMS**: ColG, Microbial Collagenase

**PHYSICAL DESCRIPTION**:
Appearance: white powder
Form: Lyophilized powder
Quality: Nickel Affinity Chromatography
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.

**PROPERTIES:**

| | |
|---|---|
| **Molecular weight** | **114,8** |
| **Optimum pH:** | **8,0** |
| **Isoelectric point:** | **5,53** |
| **Optimum Tª (ºC)** | **37** |

**COMPOSITION**:

| Identifiers | Number | Name |
|---|---|---|
| CAS number | 9001-12-1 | ColG |
| CAS number | 7647-14-5 | NaCl |
| CAS number | 77-86-1 | $(HOCH_2)_3CNH_2$ |
| CAS number | 10043-52-4 | $CaCl_2$ |

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

**ACTIVATORS**:
***PURE100 COLLAGENASE G*** is activated by four gram atom calcium ($Ca^{2+}$) per mole enzyme[2].

**INHIBITORS**:

***PURE100 COLLAGENASE G*** inhibitors include: 1,10-phenanthroline[3], 2-(1-benzyl-3-naphthalen-1-yl-ureido)-3-methyl-butyric acid[4], 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[5], 2-[benzyl(1-naphthylsulfonyl)amino]propanoic acid[6], 2-[benzyl(5-methylnaphthalene-1-sulfonyl)amino]-3-methylbutanoic acid[4], Linoleic acid [7], Linolenic acid [7] and stearic acid[7].

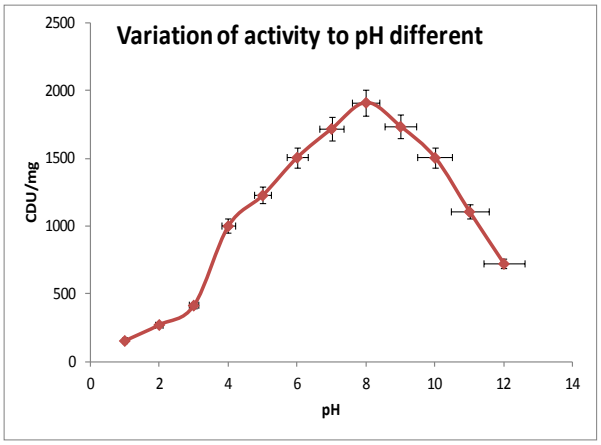
**SUBSTRATES**:

***PURE100 COLLAGENASE G*** is a soluble protein in water or aqueous buffers.
*Pure100 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.

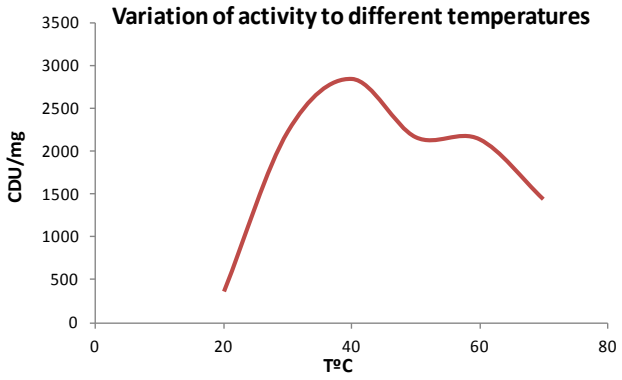
The various types of collagen are the natural substrates for Collagenase.
Many synthetic peptides have been prepared to serve as Collagenase substrates; they include: N-CBZ-gly-pro-gly-gly-pro-ala[8] (Km = 0.71 mM[9]); N-CBZ-gly-pro-leu-gly-pro[10]; N-2,4-Dinitrophenyl-pro-gln-gly-ile-ala-gly-gln-D-arg[11]; N-(3-(2-furyl)acryloyl)-leu-gly-pro-ala (FALGPA)[12]; 4-Phenylazobenzyloxycarbonyl-pro-leu-gly-pro-D-arg[13]. In addition N-Succinyl-gly-pro-leu-glypro 7-amido-4-methylcoumarin is listed as a substrate for "Collagenase-like peptidase"[14] and N-(2,4-Dinitrophenyl)-proleu-gly-leu-trp-ala-D-arg amide is listed as a substrate for "vertebrate Collagenase"[15].

**ENZYMATIC ACTIVITY AND CHARACTERIZATION**

***PURE100 COLLAGENASE G*** has an activity of >1500 CDU/mg, with Tris-HCl buffer (using collagen as substrate at 37 ºC) depending on the pH range.




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

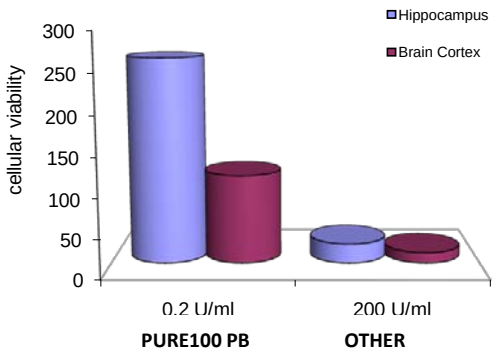



## APPLICATIONS

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




**METHOD OF PREPARATION:**

***PURE100 Collagenase G*** 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.

***PURE100 Collagenase G*** is provided in 1.000 CDU format. 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 (1.000 CDU/ml) 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**

***PURE100 Collagenase G*** 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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