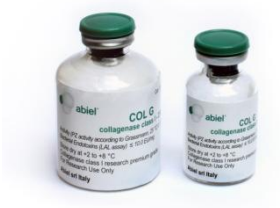
Data Sheet

Version 3.0

Revision Date: 05/09/2015

- COL G -

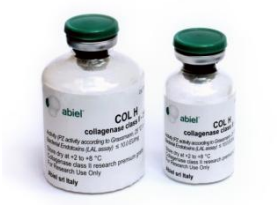
Recombinant Collagenase class I



Item No.	Item Description
001-0010	COL G, 10 mg
001-0100	COL G, 100 mg
001-0250	COL G, 250 mg

- COL H -

Recombinant Collagenase class II



Item No.	Item Description
002-0010	COL H, 10 mg
002-0100	COL H, 100 mg
002-0250	COL H, 250 mg

1. DESCRIPTION

COL G and **COL H** are recombinant collagenases (metalloproteinases) class I and class II respectively [1]. COL G and COL H are synthesized separately from *C. Histolyticum* genes by DNA recombination in *E. Coli BL21 AI* strain, bearing a Maltose Binding Protein (MBP) tag at the N-terminal end [2].
COL G and COL H are affinity chromatography purified proteins, highly pure, highly stable, lot-to-lot consistent, endotoxin-free (≤ 10 EU/mg, LAL assay) and animal-free.

CAS:	**9001-12-1**
EC:	**3.4.24.3**
Grade:	Research Premium Grade
Form:	Lyophilized white powder
Quality:	Amylose Affinity Chromatography
Inhibitors:	EDTA, EGTA, Cys, Hys, DTT, 2-mercaptoethanol
Activators:	Ca^{2+}

Their molecular weights are ~135 kDa (COL G) and ~158.5 kDa (COL H). COL G and COL H are soluble in water or aqueous buffers and express their maximum activity at **37°C, pH 7.4**.

2. SUBSTRATES

COL G and COL H play different synergic roles in collagen digestion. Indeed, COL G expresses a higher activity against **native collagen**, specifically hydrolyzing **3D-helix regions**, while COL H expresses a lower activity against the 3D helix and a higher activity against **linear collagen regions** at the motif Pro-Y-Gly-Pro [3,4]. The mix of COL G and COL H expresses a **synergic activity** that results in efficient collagen digestion [5].
For tissue dissociation, protease addition is needed to hydrolyze non-collagenous proteins and other macromolecules present in the extracellular matrix [6].

3. ENZYMATIC ACTIVITY

COL G >3.0 Units/mg* (Pz Grassmann)
COL H >30.0 Units/mg* (Pz Grassmann)

*according to Grassmann, one Unit liberates 1 µmol of Gly-Pro-Ala from Carbobenzoxy-Gly-Pro-Gly-Gly-Pro-Ala-OH (Fluka 27673) in 1 min at pH 7.4, 37 °C [7].

4. APPLICATIONS

For research use only.
Due to their high purity and specificity, COL G and COL H are especially indicated for the isolation of primary cells from liver, pancreas, heart, cartilage and stem cells from adipose tissue and others.
In these applications we recommend to use a combination of COL G and COL H in a specific activity ratio, or according to the relevant isolation protocol in order to obtain an optimal collagen digestion in cell isolation. For other applications or suggestions, contact info@abielbiotech.com or visit www.abielbiotech.com.

5. PREPARATION METHOD

We recommend to reconstitute the lyophilized COL G and COL H enzymes immediately before use in the tissue-dissociation buffer.
Reconstitute the entire vial. Do not exceed an enzyme concentration of 10 mg/ml to avoid precipitates.
Place the vial on ice and agitate gently until the enzyme is completely dissolved (about 30 min). Filter with 0.22 µm mesh for sterility.
Prepare a mix of COL G and COL H solutions in a specific activity ratio and dilute according to your protocol working solution concentration.
Add protease to the mix at 4 °C according to the specific application. Thermolysin, pronase or neutral protease/dispase can be normally used. Protease must be added immediately before use to avoid catalytic processes in the enzymatic blend. The amount of protease will define the aggressiveness of your enzyme mixture. For suggestions about your specific protocol and application please contact info@abielbiotech.com or visit www.abielbiotech.com.

6. STORAGE AND STABILITY

Lyophilized COL G and COL H are stable at -80 °C up to two years. We recommend to split in aliquots the reconstituted solutions at need and store them at -20°C up to one month or at -80°C up to 6 months.
To use aliquots later on, they can be diluted in re-constitutive buffer or can be directly added into the enzyme working solution.

▲Warning: We recommend to avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

REFERENCES

[1] Matsushita, O. et al. (1999) *J. Bacteriol.* 181(3): 923–933.
[2] Salamone, M. et al. (2012) *Chem. Eng. Trans.* 27: 259-264.
[3] Philominathan S.T. et al. (2009) *J. Biol. Chem.* 284(16) : 10868-10876;
[4] Matsushita, O. Et al. (1994) *J. Bacteriol.*176: 149-156
[5] Breite, A.G. et al. (2011) *Transplant Proc.* 43(9) : 3171-3175
[6] Salamone, M. et al. (2014) *Chem. Eng. Trans.* 38: 247-252.
[7] W. Grassmann, et al, (1960) Z. Physiol.Chemie 322:267

For suggestions about your specific protocol or application of COL G and COL H, contact us:

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