Data Sheet

Version 6.0

Revision Date: 16/01/2018

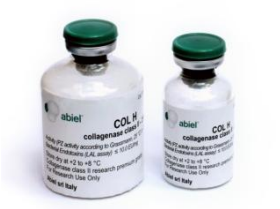
- COL G -

Recombinant Collagenase class I

Item No.	Item Description
001-0025	COL G, 25 mg ≥ 75 U
001-0100	COL G, 100 mg ≥ 300 U
001-0250	COL G, 250 mg ≥ 750 U

- COL H -

Recombinant Collagenase class II



Item No.	Item Description
002-0025	COL H, 25 mg ≥ 750 U
002-0100	COL H, 100 mg ≥ 3000 U
002-0250	COL H, 250 mg ≥ 7500 U

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 **pH 8**.

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*
COL H ≥ 30.0 Units/mg*

***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 using 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 reconstituting the lyophilized COL G and COL H enzymes in the tissue-dissociation buffer by injecting the **buffer directly into the vial**. Do not exceed an enzyme concentration of 30 U/ml (COL G) or 300 U/ml (COL H) to avoid precipitates.
Keep the vial on ice and periodically shake until the enzyme is completely dissolved. 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 splitting in aliquots the reconstituted solutions at need and storing them at -20°C up to one month or -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 avoiding 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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