*Data Sheet*

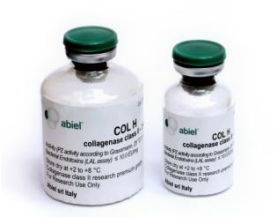
# - COL G -

## Recombinant Collagenase class I

| Item No. | Item Description |
|---|---|
| 001-001 | COL G, 75 U |
| 001-002 | COL G, 300 U |
| 001-003 | COL G, 750 U |

# - COL H -

## Recombinant Collagenase class II



| Item No. | Item Description |
|---|---|
| 002-001 | COL H, 750 U |
| 002-002 | COL H, 3000 U |
| 002-003 | COL H, 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.
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**[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:**


*Monica Salamone, PhD*
*R&D Manager*
*m.salamone@abielbiotech.com*

*Alessandra Lo Cicero, PhD*
*Product Manager*
*a.locicero@abielbiotech.com*

*Tel. +39 091 6615633*
*Fax +39 091 661 5628*