Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: info@xpressbio.com

COL G (Recombinant Collagenase Class I)
COL H (Recombinant Collagenase Class II)

For Research Use Only

CATALOG NUMBERS

CG-001 – COL G, 75 U	CH-001 – COL H, 750 U
CG-002 – COL G, 300 U	CH-002 – COL H, 3000 U
CG-003 – COL G, 750 U	CH-003 – COL H, 7500 U

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.

SUBSTRATES

COL G and COL H play different synergic roles in collagen digestion. 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].

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].

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APPLICATIONS

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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, and 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 xpressbio@xpressbio.com or visit www.xpressbio.com.

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 xpressbio@xpressbio.com or visit www.xpressbio.com.

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

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