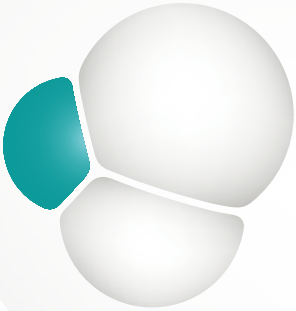


abiel®

Applicazioni Biomediche ed Industrali di Enzimi Litici

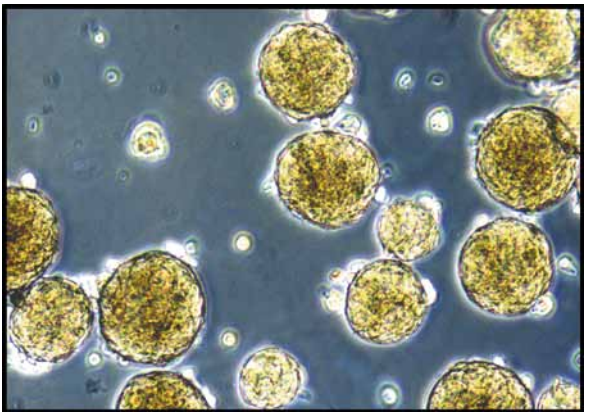
Abiel biotech

COMMITTED TO R&D PRODUCTION MARKETING OF INNOVATIVE AND HIGH QUALITY LYTIC ENZYMES

COLLAGENASE ABIEL

The new face of tissue dissociation enzymes

Innovative properties of ABIEL
recombinant collagenase G and H
for cell therapy&regenerative medicine applications



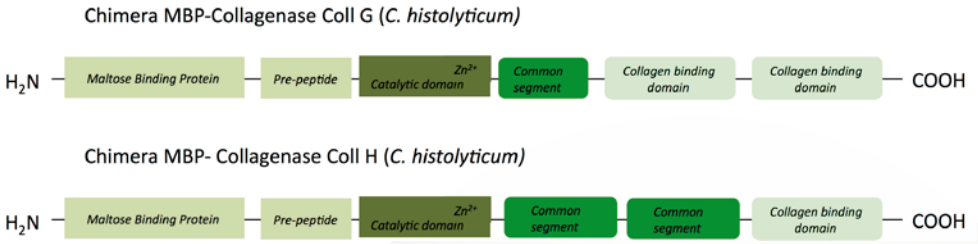
mouse pancreas islet extracted with Abiel collagenases

ABIEL PILLARS OF STRENGTH

Abiel is committed to R&D, production and marketing of innovative and high quality lytic enzymes mainly utilized in the field of tissue dissociation for cell therapy and TERM treatments.
We decided to set up the business activity because of the relevant research skills of its founders in the field of biotechnology, enzymology and biochemistry.
We supply real answer to strong market needs for pure and stable lytic enzymes, well characterized and standardized, to increase cells' yield in tissue dissociation, for cell therapy and TERM purposes.
We fulfill these unmet customer needs and currently produce by synthesis two patented collagenases COL G and COL H (G- class I and H- class II) using recombinant DNA techniques. These innovative enzymes are well standardized and characterized by a higher stability, and by a lack of toxic contaminants.
Abiel Class I and II collagenases are a research premium-grade products, utilized in all research TERM fields, as well as in clinical and industrial research centers.

COL G COL H TECHNOLOGY AND MANUFACTURING CYCLE

Abiel has been developing a key technology platform using a patented process of genetic engineering to produce by synthesis Collagenase G and H in *E. Coli's* strain. Collagenases are produced by two separate and distinct genes in *Clostridium histolyticum*; both genes have been cloned and sequenced, with generation of recombinant chimeric forms of COL G and COL H. Both gene products can be present as two or more isoforms differing in molecular weight.



FLAWLESS PROCESS CONTROL

Abiel's COL G and COL H are synthesized by fermentation, chromatographically purified, stable, highly pure and free of toxic compounds, allowing a strong competitive-edge in terms of:

- ***higher stability***
- ***complete control of the final composition of the products***
- ***high batch-to-batch reproducibility and absence of endotoxins***

New Brunswick fermentor in COL G collagenases production

ABIEL COL G AND COL H DATA SHEETS

COL G

Name of the enzyme: **MBP-COLLAGENASE G (MBP-ColG)**
Organism of origin: *Clostridium histolyticum*
Recombinant production in: *Escherichia coli* BL21 AI,
the enzyme contains a Maltose Binding
Protein (MBP) tag in its N-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: Maltose Affinity Chromatography
Storage Temperature: +2°C/+8°C
Long Term Storage Temperature: -20ºC/-80ºC

PROPERTIES:
Molecular Weight: about 135,0 kDa
Optimum pH: 7,4
Optimum T: (ºC) 37

MBP-ColG is a recombinant collagenase bears a MBP tag at the amino end. It is affinity chromatographically purified protein, highly pure, high stable, endotoxin free (≤ 10 EU/mg, LAL assay), suitable for its research application.

SUBSTRATES:
MBP-ColG is a metalloproteinase (type I), soluble in water or aqueous buffers, with capacity for hydrolyzing specifically native collagen helix regions at the motif Pro-Y- Gly- Pro.

ENZYMATIC ACTIVITY:
MBP-ColG has a Pz peptide activity >0,4 U/mg at 37°C, pH 7,4.
1U catalyzes the hydrolysis of 1µmol of Gly-Pro-Ala from Z-Gly-Pro-Ala (Flucka 27673) in 1 minute at 37°C, pH 7,4 (W. Grassmann, A. Nordwig, Z. Physiol.Chemie 322:267-1960).

APPLICATIONS:
The various types of collagen are the natural substrates for collagenases. Due to its high purity and specificity, MBP-ColG is 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 ColG/ColH in specific molar ratio in order to obtain an optimal collagen digestion.

For islets extraction from mice we strongly recommend its use in association with MBP-ColH (Abiel srl), the ratio G/H collagenases suggested is 2:1 (in total mixture of 4 mg) plus 8 µg of thermolysine or 0,2U of Neutral protease. For more details please contact Abiel srl (info@abielbiotech.com, www.abielbiotech.com).

METHOD OF PREPARATION:
MBP-ColG is available for research use in 10, 100 and 250mg formats (about 4, 40 and 100 U/ vial) as a lyophilized powder. For long term storage, we recommend to store the enzyme at -20ºC/-80ºC. We recommend dissolving the enzyme immediately before using it or to store in aliquots at -20ºC/-80°C for better preservation of the activity. We suggest avoiding multiple freeze-thaw cycles and exposure to frequent temperature changes.
To achieve the required enzymatic activity stock solution must be diluted in the re-constitutive buffer or can be directly added into the enzyme working solution.

COL H

Name of the enzyme: **MBP-COLLAGENASE H (MBP-ColH)**
Organism of origin: *Clostridium histolyticum*
Recombinant production in: *Escherichia coli* BL21 AI,
the enzyme contains a Maltose Binding
Protein (MBP) tag in its N-terminus end.

CAS: 9001-12-1

ENZYME COMMISSION NUMBER: 3.4.24.3

SYNONYMS: ColH, Microbial collagenase

PHYSICAL DESCRIPTION:
Appearance: White powder
Form: Lyophilized powder
Quality: Maltose Affinity Chromatography
Storage Temperature: +2°C/+8°C
Long Term Storage Temperature: -20ºC/-80ºC

PROPERTIES:
Molecular Weight: about 158,5kDa
Optimum pH: 7,4
Optimum T: (ºC) 37

MBP-Col H is a recombinant collagenase bears a MBP tag at the amino end. It is affinity chromatographically purified protein, highly pure, high stable, endotoxin free (≤ 10 EU/mg, LAL assay), suitable for its research application.

SUBSTRATES:
MBP-ColH is a metalloproteinase (type II), soluble in water or aqueous buffers, with capacity for hydrolyzing specifically collagen helix regions at the motif Pro-Y-Gly-Pro.

ENZYMATIC ACTIVITY:
MBP-ColH has a Pz peptide activity >1,5 U/mg at 37°C, pH 7,4.
1U catalyzes the hydrolysis of 1µmol of Gly-Pro-Ala from Z-Gly-Pro-Ala (Flucka 27673) in 1 minute at 37°C, pH 7,4 (W. Grassmann, A. Nordwig, Z. Physiol.Chemie 322:267-1960).

APPLICATIONS:
The various types of collagen are the natural substrates for collagenases.
Due to its high purity and specificity, MBP-ColH is 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 ColG/ColH in specific molar ratio in order to obtain an optimal collagen digestion.

For islets extraction from mice we strongly recommend its use in association with MBP-ColG (Abiel srl) the ratio G/H collagenases suggested is 2:1 (in total mixture of 4 mg) plus 8 µg of thermolysine or 0,2U of Neutral protease. For more details please contact Abiel srl (info@abielbiotech.com, www.abielbiotech.com).

METHOD OF PREPARATION:
MBP-ColH is provided for research use in 10, 100 and 250mg formats (about 15, 150 and 375 U/ vial) as a lyophilized powder. For long term storage, we recommend to store the enzyme at -20ºC/-80ºC. We recommend dissolving the enzyme immediately before using it or to store in aliquots at -20ºC/-80°C for better preservation of the activity. We suggest avoiding multiple freeze-thaw cycles and exposure to frequent temperature changes.
To achieve the required enzymatic activity stock solution must be diluted in the re-constitutive buffer or can be directly added into the enzyme working solution.

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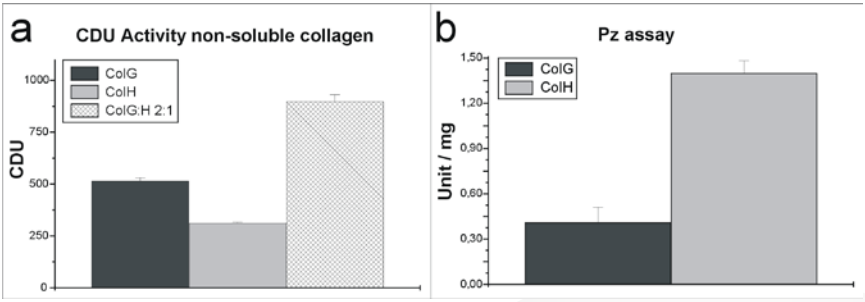
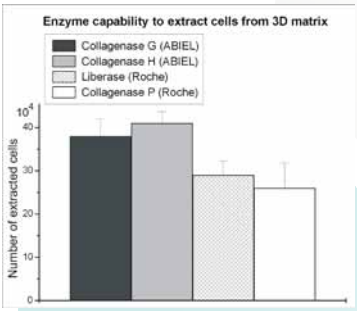
ABIEL COL G AND COL H DATA PROOF

1. ENZYMATIC ACTIVITY OF RECOMBINANT PROTEINS ON LINEAR AND THREE DIMENSIONAL SUBSTRATES.



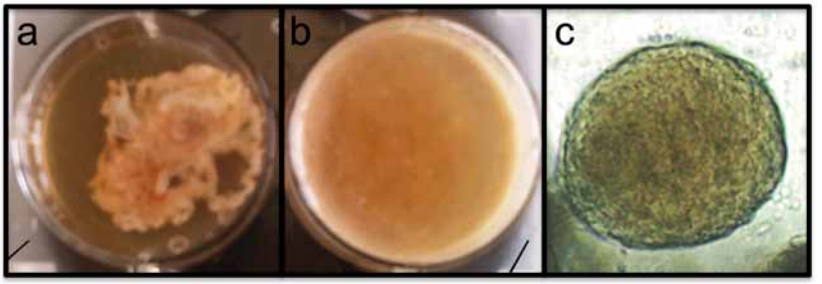
a) CDU activity of both Abiel collagenases and in the composition COL G/COL H 2:1
b) PZ evaluation of COL G and COL H activity

2. IN VITRO STUDY ON EXTRACTION CAPABILITY OF COL G AND COL H IN COMPARISON WITH LIBERASE AND COLLAGENASE P (ROCHE).



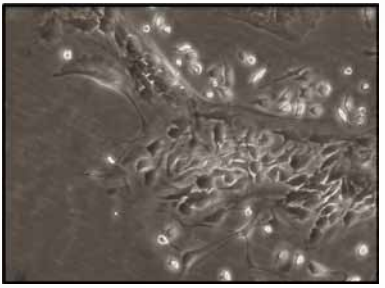
Cell extraction capability with COL G and COL H collagenases compared to Liberase and Collagenase P (Roche) when used at the same conditions.

3. EX VIVO STUDY ON EXTRACTION CAPABILITY OF COL G AND COL H (MICE LANGERHANS'S ISLETS).



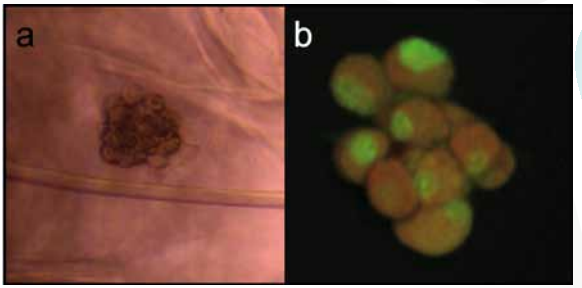
Tissue digestion of mouse pancreas using a mix of recombinant/chimera COL G/COL H 2:1, 1 mg/ml + Neutral proteases 0,2 total units
a) Non-treated mouse pancreas; b) Digested mouse pancreas; c) Purified mouse islet

4.COL G AND COL H ENZYMATIC EXTRACTION CAPABILITY OF OSTEOBLASTS FROM MOUSE.



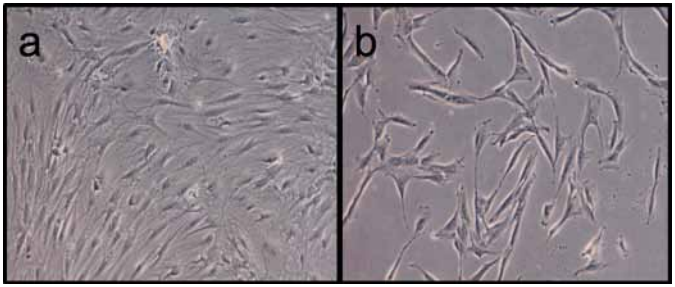
Osteoblasts isolated from mouse skull and cultured in presence of ascorbic acid

5. EX VIVO STUDY ON EXTRACTION CAPABILITY OF COL G AND COL H OF BOVINE HOOF CONDROCYTES.



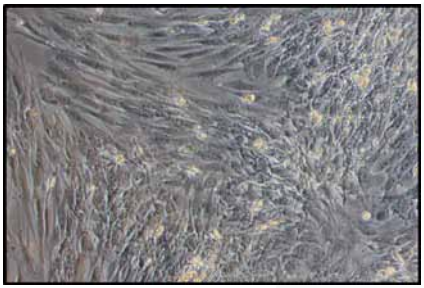
a) Condrocytes from bovine hoof, CMC hydrogel cultured
b) Condrocytes dyed with OA/ EtBr

6. COL G AND COL H ENZYMATIC EXTRACTION CAPABILITY OF HUMAN FIBROBLASTS.



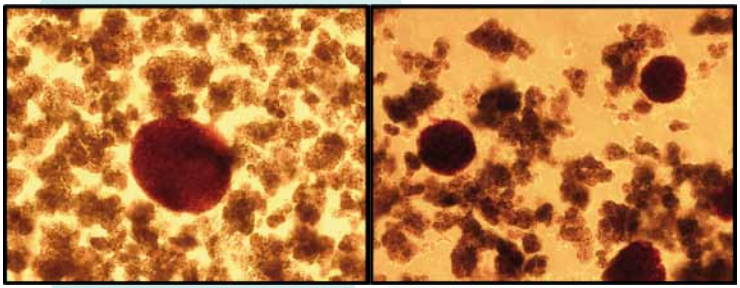
Abiel COL G and COL H (a) and Sigma(b) collagenases comparison in human dermal capability extraction, 8 days

7.COL G AND COL H ENZYMATIC EXTRACTION CAPABILITY OF STEM CELLS FROM ADIPOSE TISSUE.



Mesenchimal stem cells from human adipose tissue obtained using Abiel enzymes

8. COL G AND COL H ENZYMATIC EXTRACTION CAPABILITY OF ISLETS FROM DONOR PANCREATA.



Islets from human pancreata tissue

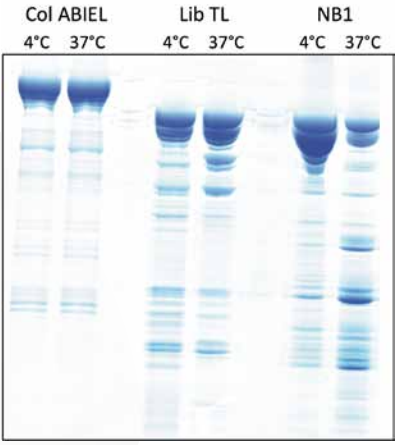
ABIEL COL G AND COL H BENEFITS FOR THE CUSTOMERS

Abiel's enzymes, manufactured by synthesis, show strong improvements in terms of

- ***higher stability***
- ***complete control of the final composition of the products***
- ***high batch-to-batch reproducibility and absence of endotoxins***

STABILITY OF COL G AND COL H

Collagenase ABIEL mix GH, Thermolysin TL and Collagenase Serva NB1 have been incubated for 1 hr at 37°C and then loaded in SDS-PAGE to compare the pattern with the control. As showed in the picture the ABIEL mix GH shows at 4° and 37°C the same pattern to confirm the major stability.



COL G AND COL H: ENDOTOXIN CONTENT, PERCENTAGE OF VARIATION BETWEEN BATCHES AND AMOUNT OF ENZYME ACTIVITY

MEAN CHANGE BETWEEN ABIEL RECOMBINANT COLLAGENASE CLASS I AND II BATCHES						
	Col G			Col H		
	Mean	Max	Min	Mean	Max	Min
*Endotoxins content EU/mg**	2,5	3,0	2,2	2,1	2,4	1,8
*Percentage variability in composition between batches***	+/- 1,6	+/- 2,16	+/- 1,01	+/- 1,5	+/- 1,68	+/- 1,03
*Percentage of enzyme activity present in the lyophilized****	92,35	94,87	90,76	91,16	92,39	89,76

** Endotoxin evaluation was performed using LAL assay*

*** The percentage of variation between batches was obtained by densitometric analysis (Image 4.12 NIH program) of the profiles of the purified enzymes separated by SDS-PAGE*

**** Percentage amount of enzyme activity was determinated by SDS-PAGE and gelatin zymography profiles comparison. In each kind of evaluation were compared almost eight different batches.*

EXCELLENCE THROUGH RESEARCH & DEVELOPMENT

ABIEL R&D PROJECTS AND SERVICES

We strongly pursuing the following R&D activities to implement our growth and sustainable programs:

1.To characterize and purify proteolytic enzymes from marine organisms, able to work at low temperatures (<20°C) therefore preserving the structural and functional characteristics in the processes where they are applied.
2.To generate other recombinant enzymes, obtained using particular techniques of molecular biology able to produce such enzymes in an high quantity and pure fractions.

ABIEL PROVIDES SEVERAL SERVICES IN THE FIELD OF LYTIC ENZYMES

- Screening and characterization of lytic enzymes from biomarine and other natural resources using natural and synthetic substrate.
- Engineering and development of recombinant proteins characterized by higher solubility, stability and activity.
- Production of enzymes in fermenters and their purifications.
- New in vitro test to assess the toxicity of other marketed enzymatic procedures to isolate and purify human cells for TERM treatments.
- In vivo assays (mouse and rat model) to select enzyme for tissue dissociation.

COLLAGENASE ABIEL

The new face of tissue dissociation enzymes

innovative properties of ABIEL recombinant collagenase G and H for cell therapy&regenerative medicine applications

COL G and COL H benefits for the customers

Abiel's enzymes, show strong improvements in terms of:

- ***higher stability***
- ***complete control of the final composition of the products***
- ***high batch-to-batch reproducibility and absence of endotoxins***

We assist our customers with their projects every step of the way.
Further details of COL G and COL H are provided at:
www.abielbiotech.com

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