Recombinant Collagenases (COL G and COL H) for cell isolation

1. Background

Collagen is the main structural protein of mammalian extra-cellular matrix, and it is present in different isoforms. All collagen molecules share a triple-stranded helical structure, resistant to proteases, which **can only be degraded by specific collagenases.**

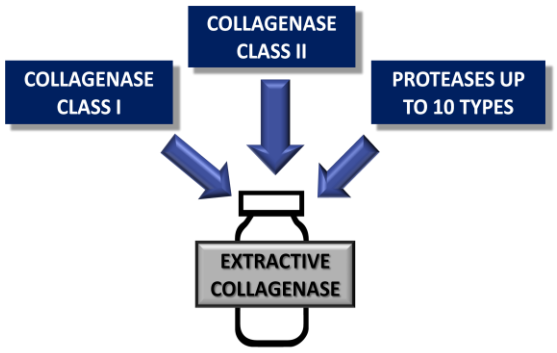
The gold standard for performing cell isolation is the enzymatic digestion of the target tissue/organ by collagenases. Among collagenases the most important ones are **classes I and II** (isoforms I and II according to the nomenclature of Bond and Van Wart 1984 [1]), expressed by *C. histolyticum*, which are preferred to others, since they are **proteolytically active** practically on **all mammalian collagen isoforms**.

Collagenases

• **Class I**
High collagenolytic activity, specifically hydrolyzing native collagen 3D-helix regions
• **Class II**
modest activity against 3D collagen helix, acting on linear collagen regions at the motif Pro-Y-Gly-Pro [4].

2. Collagenases extracted from *C. histolyticum*

The current technologies to produce collagenases for research and biomedical use are based on the culture of *C. histolyticum* and the subsequent protein purification thereof. The resulting 'Collagenase' is actually a **"blend"**, containing different percentage/weight ratios of the two collagenase isoforms (class I and class II), plus an ill-defined number of other proteases (clostripain, trypsin like, caseinase activity, etc.) [5].



Extractive Collagenases
Limits in Cells Isolation

• **Variability in composition** of class I and II enzymes.
• **Lower stability** since the presence of proteases.
• **High variability in the efficiency** of cell extraction and therefore low **reproducibility** that hampers standardization of protocols.
• **Residual trypsin-like activity** despite improved purification methods.

The successful outcome of cell isolation strongly depends on activity, purity, and formulation of 'Collagenase' [4-5]. However, the extraction process of the 'Collagenase' blend from *C. hystolyticum* makes it **difficult to obtain reproducible batches**, while the presence of the proteases can contribute to a **lower stability** due to enzymatic autocatalytic processes. Low batches consistency and low stability can lead to low reproducibility and low standardization of protocols for cell isolation [6].

3. Abiel's Recombinant Collagenases

Abiel's collagenases Class I (namely Col G) and Class II (namely Col H) of *C. histolyticum* are cloned and expressed in *E. Coli* and the tagged recombinant proteins are purified by affinity chromatography. Separate productions of the enzymes (animal-free) ensures **high yield** (>1 g/litre) and **high purity** (>99%) of COL G or COL H enzyme preparations with excellent **batch-to-batch reproducibility** [7].

In contrast to collagenase extracted from *C. histolyticum*, Abiel's recombinant products allow to prepare **customized** and **standardized mixtures** [8]. These mixtures contain a defined ratio of the two collagenases (COL G and COL H) and a defined protease. Thus, the blending is based on activity determination and not on weight ratios with ill-defined composition and activity. Taken together, the separate production allows defining the precise enzymatic activity of the final blend adapted to the use in a specific cell isolation procedure.

For cell isolation, one generic protease is required to digest the non-collagenous part of the ECM. Mixing COL G + COL H + protease, an efficient ECM digestion is obtained [9].

*** either thermolysin, neutral protease/dispase, pronase. For your specific application please refer to** info@abielbiotech.com

Benefits of Abiel's enzymes

- **Reproducible batches:** avoid batch-to-batch variation.
- **Consistency:** consistent results from each experiment. Time saving–cost saving.
- **Pure collagenase (>99%):** no contaminating proteases, which reduce over-digestion.
- **Reagent Flexibility:** control collagenase digestion rate (or aggressiveness) adapted to protocol requirements.
- **High stability** (up to 3 years): Lyophilised product stable at RT or cold storage; no autodegradation by proteases.
- **Endotoxin-free**: No off-shoot toxic effects on cells.

Applications

Human
- Langerhans' islets
- Fibroblasts (dermis regeneration)
- Mesenchymal stem cells (adipose tissue)
- Stem cells (diff. tissues)

Rat
- Langerhans' islets

Mouse
- Hepatocytes (liver regeneration)
- Mesenchymal stem cells (adipose tissue)
- Osteoblasts (bone regeneration)

Bovine
- Chondrocytes (cartilage regeneration)

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

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