

Product No. 892 431: 1 vial

Product No. 892 432: 2 vials

Purified recombinant collagenase  
Brightase-C (40 mg/vial)

Product Description:

*Grimontia hollisae* strain 1706B, which produces a single collagenase with a very high specific activity, was isolated from seashore sand collected from the Shin-Kiba coast in Tokyo<sup>1)</sup>. Brightase-C, the recombinant collagenase from *G. hollisae*, is produced with stable activity using the *Brevibacillus* expression system<sup>2)</sup>. This system leads to the expression of recombinant proteins with low endotoxin contamination since *Brevibacillus* is a Gram-positive bacterium. Our purified collagenase digests only various types of collagen and doesn't have any neutral proteinase activity. Due to no neutral proteinase activity in Brightase-C, it is necessary to add Brightase-TH (recombinant thermolysin) for dissociation of tissues to isolate primary cells.

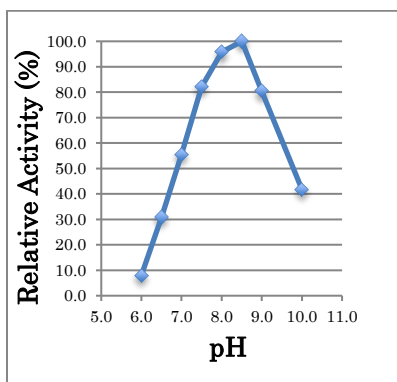
Content:Recombinant collagenase from *Grimontia hollisae* (lyophilized): 40 mg/ vial

※ Lyophilized at 20 mg/ml of 2 mL solution

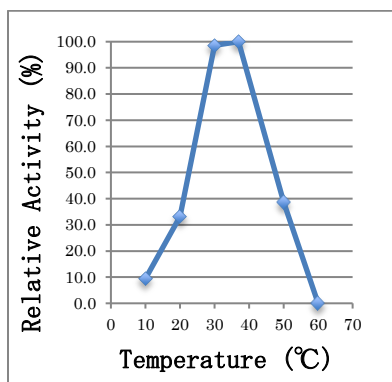
(HEPES buffer: 20 mM HEPES, 30 mM NaCl, 2 mM CaCl<sub>2</sub>, pH 7.5)※ Organism of origin: *Grimontia hollisae*<sup>3)</sup>※ Recombinant production in: *Brevibacillus choshinensis*<sup>2)</sup>(Endotoxin-free ( $\leq 10$  EU/mg), animal-derived components free)Specification

Activity*	: $\geq 6,000$ U/ mg	
Optimal pH	: 7.5-8.5	(Fig.1)
Optimal temperature	: 30~40 °C	(Fig.2)
Inhibitors	: EDTA, EGTA, o-phenanthroline	
M.W.	: 61,900	
E <sup>1%</sup> <sub>1cm</sub> (280nm)	: 18.1 (pH7.5)	
Content	: 40 mg/ vial	
Endotoxin	: $\leq 10$ EU/ mg	

\*One unit of collagenolytic activity was defined as the amount degrading 1  $\mu$ g of FITC-labeled collagen at pH7.5 at 30°C per min.



(Fig. 1)



(Fig. 2)

### Storage condition

Brightase-C should be stored at under -20 °C and is stable for 2 years at this condition. After dissolving a lyophilized product, the solution should be stored in aliquots at under -20 °C. Please avoid multiple freeze-thaw cycles.

### Stock solution

Stock solution of Brightase-C can be prepared in 2 mL sterile distilled water by agitating at 4°C for 30 min. It contains 20 mg/ml enzyme, 20 mM HEPES, 30 mM NaCl and 2 mM CaCl<sub>2</sub> (pH 7.5) after reconstitution.

### Working solution

The working solution is prepared by mixing the cold enzyme stock solution with cold buffer (containing  $\geq 1$  mM CaCl<sub>2</sub>). Calcium is necessary for activity of collagenase. If sterile filtration is desired, a sterile filter with low protein binding properties should be used.

### References

- 1) Suzuki, K., and Matsubara, Y., *Hikakukagaku*, **44**, 64–71 (1998)
- 2) Mizukami, M. et al., *Current Pharmaceutical Biotechnology*, **11**, 251-258 (2010)
- 3) Teramura, N. et al., *Journal of Bacteriology*, **193**, 3049-3056 (2011)

### Contact and Support

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