RESEARCH USE ONLY Ver. 001

Product No. 892 441: 1 vial
Product No. 892 442: 2 vials

Purified recombinant thermolysin
Brightase-TH (4.7 mg/vial)

Product Description:

Brightase-TH, the recombinant thermolysin from *Bacillus thermoproteolyticus rokko*[1], is produced with stable activity using the *Brevibacillus* expression system[2]. This system leads to the expression of recombinant proteins with low endotoxin contamination since *Brevibacillus* is a Gram-positive bacterium. Our purified thermolysin has typical neutral proteinase activity. Due to no collagenolytic activity in Brightase-TH, it is necessary to add Brightase-C (recombinant collagenase) for dissociation of tissues to isolate primary cells.

Content:

Recombinant thermolysin from *Bacillus thermoproteolyticus rokko* (lyophilized): 4.7 mg/ vial

※ Lyophilized at 0.94 mg/ml of 5 mL solution
(HEPES buffer: 50 mM HEPES, 2 M NaCl, 5 mM $CaCl_2$, pH 7.5)
※ Organism of origin: *Bacillus thermoproteolyticus rokko*[1]
※ Recombinant production in: *Brevibacillus choshinensis*[2]
(Endotoxin-free (≦10 EU/mg), animal-derived components free)

Specification

EC number	: 3.4.24.27
Activity*	: 0.8~1.2 MOCAc U/ mg
Optimal pH	: 7.0~7.5
Recommended temperature for use: 30～40 ℃	
Thermal stability	: 80% of activity remains after enzyme is incubated in HEPES buffer at 70°C for 2 hours.
Inhibitors	: EDTA, phosphoramidon
M.W.	: 34,600
$E^{1\%}_{1cm}$ (280nm)	: 17.65 (pH7.0)
Content	: 4.7 mg/ vial
Endotoxin	: ≦ 10 EU/ mg

*One unit of activity was defined as the amount of enzyme releasing 1 μM of MOCAc-PLG from synthetic substrate (MOCAc-PLGL(Dpa)AR) at pH 7.5 at 30°C per sec. [3, 4]

Storage condition

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

Stock solution

Stock solution of Brightase-TH can be prepared in 5 mL sterile distilled water by agitating at 4℃ for 30 min. It contains 0.94 mg/ml enzyme, 50 mM HEPES, 2 M NaCl and 5 mM $CaCl_2$ (pH 7.5) after reconstitution.

Working solution

The working solution is prepared by mixing the cold enzyme stock solution with cold buffer (containing ≧ 1 mM $CaCl_2$). Calcium is necessary for activity of thermolysin. If sterile filtration is desired, a sterile filter with low protein binding properties should be used.

Measurement of enzyme activity

Brightase-TH has a MOCAc-PLGL(Dpa)AR degrading activity, 0.8~1.2 MOCAc U/ mg at pH7.5 at 30°C per sec[3, 4]. One MOCAc unit is comparable to 100 caseinolytic activity (CU). One CU will hydrolyze casein to produce color equivalent to 1.0 μmol of tyrosine at pH7.5 at 37°C per min (Color by Folin-Ciocalteu regent) [5].

References

1) O'Donohue, MJ. et al., *Biochemical Journal,* **300**, 599–603 (1994)
2) Mizukami, M. et al., *Current Pharmaceutical Biotechnology*, **11**, 251-258 (2010)
3) Muta, Y. and Inouye, K., *Journal of Biochemistry*, **132**, 945-951 (2002)
4) Oneda, H. et al., *Biosci. Biotechnol. Biochem*., **68**, 1811-1813 (2004)
5) Anson, ML., *J. Gen Physiol*, **22**, 79-89 (1938)

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