Enteropeptidase/Enterokinase Cleavage Kit

LS-K671-100 (100 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Introduction

Enteropeptidase (Enterokinase, EC 3.4.21.9) is a serine protease involved in activation of trypsinogen to trypsin, which in turn results in the activation of various digestive enzymes. It recognizes a highly specific amino acid sequence 'DDDDK' and cleaves after the lysine residue (K). The high specific activity of Enteropeptidase has been utilized in cleaving a variety of native or fusion proteins containing the above recognition motif. LSBio's Enteropeptidase Cleavage Kit contains highly active light chain fragment of human Enteropeptidase. The pure enzyme is an excellent tool to obtain a wild type protein sequence from a fusion protein, containing Enteropeptidase recognition sequence (DDDDK). This Enteropeptidase cleavage kit is sufficient for cleaving at least 5 mg of target protein. The residual enteropeptidase left in the reaction mix will not interfere with most of the downstream applications. Following cleavage of the target protein, Enteropeptidase can be removed using Agarose beads.

Applications

- Efficiently removes tags from recombinant fusion proteins containing accessible Enteropeptidase-specific recognition sequence.

Components

Component	K671-100 100 Tests	Cap Code
Enteropeptidase Cleavage Buffer	20 ml	WM
Human Enteropeptidase (100 U)	34 µl	Green
Cleavage Control Protein (hPRL-Trx) (50 µg)	1 vial	Blue

Materials Not Supplied

- Sterile Eppendorf tubes or Falcon tubes.
- 50% glycerol.

Storage Conditions and Reagents Preparation

Store kit at -20°C. Warm Enteropeptidase Cleavage Buffer to room temperature before use. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- Human Enteropeptidase: Add 66 µl of 50% glycerol to the vial containing Enteropeptidase to make 1 U/µl Enteropeptidase solution. Aliquot & keep at -80°C for long term storage. Avoid repeated freeze/thaws. Use within two months.
- Cleavage Control Protein: Reconstitute with 50 µl of Enteropeptidase Cleavage Buffer to obtain 1 mg/ml Control Protein solution.

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Procedure

a) Target Protein: Dilute your target fusion protein to final concentration of 1 mg/ml with appropriate volume of Enteropeptidase Cleavage Buffer.

b) Reaction Mix: In a sterile Eppendorf tube, mix enough of the following reagents for the number of cleavage reactions to be performed:

	Target Protein Mix (50 µg)	Cleavage Control Protein Mix (10 µg)
Target Protein	50 µl	-
Control Protein	-	10 µl
Human Enteropeptidase (1 U/µl)	1 µl	0.2 µl

Mix gently by pipetting up and down (do not vortex) and gently agitate at room temperature for 20 hours. Aliquot ~10 µl (10 µg) from the target protein reaction mixture at regular time intervals and freeze at -20°C. After 20 hours, collect all the samples and analyze by SDS-PAGE. For the Cleavage Control Protein, run 5 µl of undigested Cleavage Control Protein along with the digested Cleavage Control Protein mix (5 µl) after 20 hours.

Note: In order to find the optimum cleavage conditions, it is recommended to run preliminary digestion reactions at a small scale. For that, the enzyme stock solution (1 U/µl) can be further diluted with the Enterokinase Cleavage Buffer to obtain an enzyme solution containing 0.01, 0.05, 0.08, 0.1, 0.5 and 0.8 U. Successful cleavage with the Enteropeptidase is dependent upon proper folding of the fusion protein allowing for easier exposure of the enzyme recognition sequence. Once optimum cleavage conditions are obtained, the reaction can be scaled up to digest the entire amount of the target protein.

One unit of Enteropeptidase is the amount of the enzyme required to cleave 50 µg of the Control Protein (hPRL-Trx) within 20 hours at 23°C to 95% completion.

Sample Data

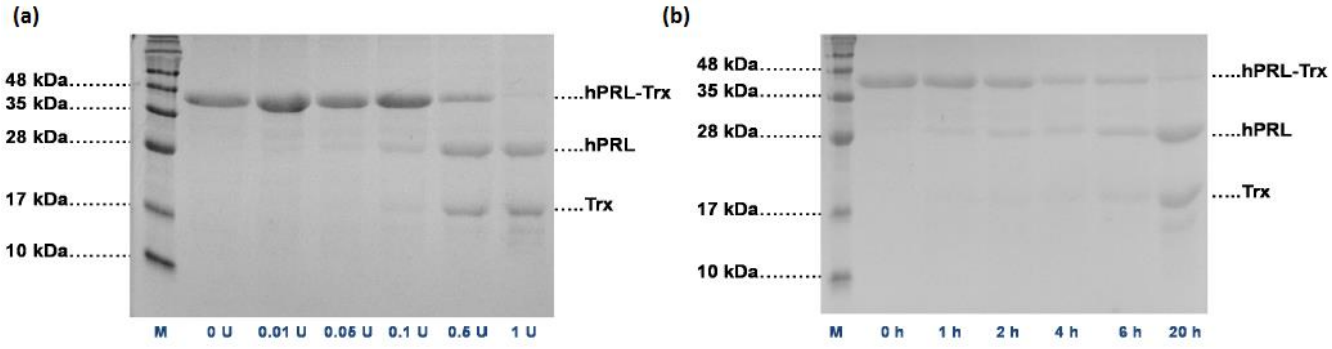

Figure: SDS-PAGE analyses of hPRL-Trx (42.3 kDa): hPRL-Trx (50 µg) was digested into its individual protein fragments hPRL (25.3 kDa) and Trx (17 kDa) using different amounts (0.01-1 U) of Enteropeptidase at RT for 20 hours (a). Analysis of hPRL-Trx digestion at different time points at RT using 1 U of Enteropeptidase (b).

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LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com