Recombinant Bovine Enterokinase / Enteropeptidase

Catalog Number: SEKP01

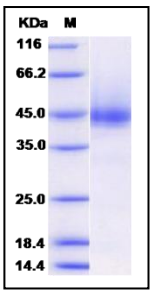
General Information

Synonym: EK, Enterokinase, PRSS7, Enteropeptidase, EC 3.4.21.9, Serine protease 7, ENTK, MGC133046.
Protein Construction: A DNA sequence encoding the light chain (Ile 801-His 1035) of bovine Enterokinase(NP_776864)was expressed.
Expression Host: Yeast (Pichia pastoris)
Content：

Name	Description	Amount
EK protease	20mMTris, 200mMNaCL, 10%Glycerol, 5%Mannitol, 5%Trehalose,pH7.2	100U
Cleavage Control Protein	Lyophilized from a 0.2 μm filtered solution in buffer PBS,	50μg
10X rEK Storage/Reaction Buffer	500 mM NaCl, 200 mM Tris-HCl, pH 7.2	2mL
Sterile Deionized Water	Detailed the lyophilized products	1mL

QC Testing

Purity: > 95%, as determined by SDS-PAGE and SEC-HPLC Analysis
Endotoxin: < 1.0 EU per 1 μg of the cytokine as determined by the LAL method
Stability: The enzymes can be used for up to twelve months stored at -70℃
Predicted N terminal: Ile 801
Molecular Mass: The recombinant bovine Enterokinase consists of 234 amino acids and has a calculated molecular mass of 26.1kDa. As a result of glycosylation, the recombinant protein migrates as an approximately 45kDa protein in SDS-PAGE under reducing conditions.
SDS-PAGE:



Usage Guide

Storage:

Shipped at room temperature. The liquid or lyophilized enzyme is stable for at least 21d when stored at 37℃ (or room temperature).
Store it under sterile conditions at -70℃ upon receiving. Recommend to aliquot the protein into smaller quantities for optimal storage. Avoid repeated freeze-thaw cycles.

Reconstitution:

Resuspend the enzyme powder with sterile water. Keep reconstituted enzyme at -20℃ in aliquots.

An example to estimate the appropriate usage of the enzyme：

One unit is defined as the amount of enzyme needed to cleave 50μg of control fusion protein for 16 hours at 20℃ in a buffer of 20 mM Tris-HCl, 50 mM NaCl, pH 7.2.
The enzyme cleavage condition will be adjusted for different fusion proteins. It is recommended to test several Enterokinase concentrations, temperatures and incubation times to optimize specificity and efficiency of cleavage. The following protocol is an example to find out appropriate conditions.

Experimental design

Enterokinase was diluted with 200μL of water and Control protein was diluted 20μL of water too. Then added 20μL of Control protein, 2μL of Enterokinase, 5μL of 10X reaction buffer, and 23ul deionized water into a microcentrifuge tube (1.5mL). Incubate the reactions at room temperature (20-21℃) for 16h.
Take 10 μL of mixture sample and add 10 μL of 2X concentrated SDS-PAGE buffer. The sample is boiled for 5min and the cleavage effect was analyzed by SDS-PAGE electrophoresis under reducing conditions.

Table 1: Enterokinase reaction system

Substance	Amount
10X cleavage buffer	5μL
Target protein	20μL
Diluted Enterkinase	2μL
Deionized water	23μL
Total	50μL

The recommending ratio of enzyme and substrate is about 1U/50μg to 100μg. But for each fusion protein the ratio is different, it depends on pilot experiment to find out the suitable conditions.

Recombinant Bovine Enterokinase / Enteropeptidase

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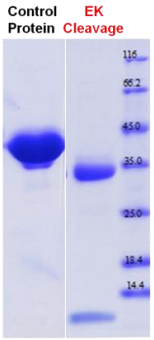
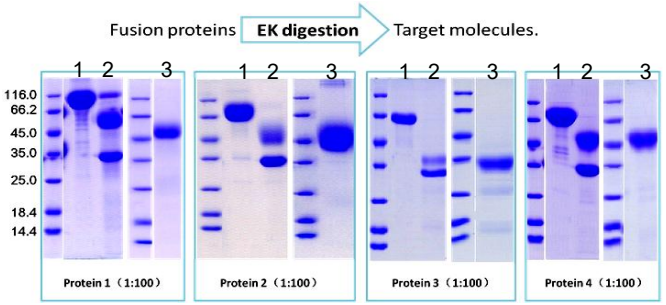
Usage of cleavage control protein



Examples for cleavage of fusion proteins

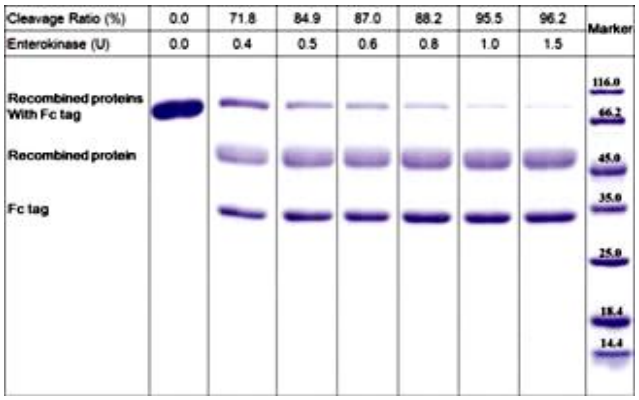
Note：Each target protein presents the cleavage site somewhat differently, it is recommended to test several enterokinase concentrations, temperatures and incubation times to optimize specificity and efficiency of cleavage.

Example 1.



1.Fusion protein
2.1:100 Enterokinase digestion
3.Target protein

Example 2.



Protein Description

Enterokinase is a member of the trypsin family of serine proteases. The precursor protein is cleaved into two chains which then forms a heterodimer linked by a disulfide bond. The heavy chain anchors enterokinase in the intestinal brush border membrane and the light chain is the catalytic subunit, which initiates conversion activation of a subset of pancreatic proteolytic proenzymes. Enterokinase is the physiological activator of trypsinogen and has a specificity for the sequence (Asp)4-Lys-Ile. The mature trypsin in turn activates other proenzymes including chymotrypsinogen, procarboxypeptidases, and proelastases. In addition, Enterokinase is a tool protease widely utilized in the cleavage of recombinant fusion proteins.

References

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4 Collins-Racie, L.A. et al., 1995, Biotechnology. 13: 982-957.

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