

User Manual

Version 1.0

Product name: Enterokinase

Cat #: EK-100, EK-200, EK-OEM

Description:

Enterokinase (EK) is an enzyme produced by cells of the duodenum and involved in human digestion. It plays a role of turning trypsinogen to its active form trypsin, and indirectly activates the pancreatic digestive enzymes. Enterokinase is a specific protease that cleaves after a lysine preceded by four aspartic acids: Asp-Asp-Asp-Asp-Lys. Enterokinase will not work if the recognition site is followed by a proline.

Recombinant Bovine Enterokinase (His-tagged) (rbEK) as the light chain is a single glycosylated polypeptide chain containing 200 amino acids and a 6 × His at C-terminus.

Source

E. coli

Species

Bovine

Purity

> 95 % as determined by SDS-PAGE

Endotoxin

< 1 EU per µg of the protein as determined by the LAL method

Unit Definition

One unit is defined as the amount of enzyme needed to cleave 50 µg of fusion protein in 16 hours to 95% completion at 22°C in a buffer containing 25mM Tris-HCl, pH 8.0.

Biological Activity

100 IU/µg

Storage

rbEK remains stable up to 1 year at -20°C from date of receipt. It will remain stable at 37°C for one week without losing any activity. Please avoid freeze-thaw cycles. Avoid repeated freeze-thaw cycles.

Protocol

EK Digestion

One unit of EK could cleave 50 µg of fusion protein in 16 hours to 95% completion at 22°C. However, because each target protein has the different position of cleavage site, it is recommended to optimize the concentrations of EK, incubation times and temperatures in order to find the best cleavage condition.

Typical reaction conditions are as follows:

Combine 25 ug of sample with reaction buffer and H₂O (if necessary) to a total reaction volume of 20 uL

* Recommended Reaction Buffer: 20 mM Tris-HCl, 50 mM NaCl, 2 mM CaCl₂ (pH 8.0)

Add 1uL of Enterokinase light chain

Incubate at 25°C for 16 hours

Notes: Enterokinase is inhibited by high salt concentrations. For optimal activity NaCl concentration should be 100mM or less. The pH of the buffer should be between 6 and 9. The enzyme requires 2 mM Calcium for activity.

Scale up

Scale up the reaction proportionality according to the best cleavage result.