Enterokinase

Cat. No. : RK20553

ABclonal Technology

Product components

Components	Component number	Size-1 480 U	Size-2 2400 U
Enterokinase (16,000 U/mL)	RM20537	30 μL	150 μL
10X EK Reaction Buffer	RM20809	1 mL	1 mL*5

Product Description

Enterokinase is a specific protease that cleaves after lysine at its cleavage site Asp-Asp-Asp-Asp-Lys. It will sometimes cleave at other basic residues, depending on the conformation of the protein substrate. This product is a short chain of bovine enterokinase expressed in mammalian cells, with a theoretical molecular weight of about 29 kd, and is purified by Strep tag affinity.

Applications

Removal of labeled peptides from N-terminal and Met-N-terminal fusion proteins;

Protein modification and amino acid sequence determination

Store

-20℃

Definition of Activity Unit

1 unit is defined as the amount of enzyme required to cleave 25 μg of a specific substrate containing the EK enzyme cleavage site (DDDDK) to 95% completion in 16 hours at 25°C in a total reaction volume of 25 μl.

Storage Buffer

20 mM Tris-HCl, 200 mM NaCl, 2 mM $CaCl_2$, 50% Glycerol, pH 7.2 @ 4°C

Protocol

1. Add 25 μg of sample to the system, add 2 μL of 10×EK Reaction Buffer, and mix gently with ultrapure water to 19 μL.
2. Add 1 μL of Enterokinase.
3. Mix the reaction components and centrifuge briefly to collect the solution to the bottom of the tube.
4. Incubate the mixture at 25 °C for 16 h.

Note: Enterokinase can be diluted directly to the appropriate concentration using 10×EK Reaction Buffer. Enterokinase is inhibited by high salt concentrations. For optimal activity, NaCl concentrations should be 100mM or less. The pH of the buffer should be between 6 and 9. The enzyme requires 2 mM calcium to be active.

Precautions

1. 250 mM NaCl reduced enterokinase activity to 75% of normal and 2 M NaCl almost completely inhibited enzyme activity (Barratti et al., 1973). In addition, enzyme activity is also inhibited in the following environments (urea > 2 M, β-mercaptoethanol (β-ME) > 20 mM, SDS > 0.1%, imidazole > 50 mM, and pH < 6 or PH > 9).
2. The optimal incubation time and enzyme concentration for a particular substrate must be determined empirically, as the cleavage efficiency of Enterokinase is altered by the adjacent steric hindrance of the surrounding plasma site at different egg sites.