Recombinant Bovine Enterokinase Kit
Cat. No.: Z03004

I. 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. The product of Z03004 is the light chain of recombinant Bovine Enterokinase that is a single glycosylated polypeptide chain containing 200 amino acids. A fully biologically active molecule, rbEK has a molecular mass of 22.7kDa. rbEK with 6 × His-tag binds with Ni2+ affinity chromatography and was designed for removing from digestion system.

II. Components

- 100 IU(or 500IU or 5000IU) Recombinant Bovine Enterokinase (in 20mM Tris-HCl, pH 7.4, 200mM NaCl, 2mM $CaCl_2$, 50% glycerol)
- 100μg Cleavage Control Protein (Lyophilized after extensive dialysis against PBS, pH 7.0)
- 3.6 ml EK Dilution/Storage Buffer (20mM Tris-HCl, pH 7.4, 200mM NaCl, 2mM $CaCl_2$, 50% glycerol)
- 1.8 ml 10X EK Cleavage/Capture Buffer (200mM Tris-HCl, pH 7.4, 500mM NaCl, 20mM $CaCl_2$)

Storage: Store kit components at –20°C.

III. Dialysis of the Fusion Protein

If the buffer of fusion protein contains the following reagentes, it is necessary to dialyze your fusion protein before the digestion.

1. > 2M urea, > 250mM NaCl, > 20mM β -mercaptoethanol, >0.1% SDS, >1% Triton X-100, or > 50 mM imidazole.
2. pH values below 6 and above 9.
3. Avoid the presence of serine protease inhibitors.

IV. EK Digestion

One unit of EK could cleave 50μg 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.

Small scale optimization

For the small cleavage, EK can be diluted using EK Dilution/Storage Buffer. The following is an example of optimizing the cleavage condition.

1. Make 4 serial dilution of Enterokinase (5 IU/μl) in EK Dilution/Storage Buffer (0.001 IU, 0.01 IU, 0.1 IU, and 1 IU enzymes per μl).
2. Set up 5 reactions, including a reaction without EK for the control:

Fusion Protein	10 μg
10X EK Cleavage/Capture Buffer	5 μl
Diluted EK(add 2 μl EK Dilution/Storage Buffer for the control)	2 μl
Deionized Water	x μl
Total volume	50 μl

3. Mix well and incubated at 22°C of 1h, 3h, 5h and overnight (~16 hours).
4. Load 10 μl on an SDS-PAGE gel to determine the best cleavage result. If the result is unsuitable, the optimization of temperatures can be tested.

Small up

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

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