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# Recombinant Enterokinase

**Product Number: RPE110**

## Shipping and Storage

1. Stable for 24 months at -20℃ or below,
2. No activity loss for up to 1 week at 25℃,
3. Buffer Composition: 50mM Tris-HCl (pH 8.0), 250mM NaCl, 2mM $Ca^{2+}$, 50% glycerol,
4. Freeze-Thaw Stability: No activity loss after up to 5 repeated freeze-thaw cycles,
5. Transportation: Stable when shipped with blue ice (cold chain transport).

## Component

| Component | RPE110-100U | RPE110-500U | RPE110-1000U | RPE110-10KU | RPE110-100KU |
|---|---|---|---|---|---|
| Recombinant Enterokinase | 100U | 500U | 1000U | 10KU | 100KU |

## Description

Recombinant Bovine Enterokinase is a high-purity recombinant fragment of the bovine enterokinase light chain. Purified by HPLC, it exhibits exceptional purity and specificity, and is free of other contaminating proteases. Enterokinase specifically cleaves the peptide bond at the carboxyl terminus of lysine in the sequence: Asp-Asp-Asp-Asp-Lys (DDDDK). It efficiently cleaves fusion proteins over a broad pH range (4.5–9.5) and a wide temperature range. This enzyme is used to remove N-terminal fusion tags from recombinant proteins to obtain the target protein of interest.

## Feature

1. Source: Recombinant E. coli expression
2. Appearance: Clear, colorless to pale yellow liquid
3. Activity: ≥ 5.0 U/μL
4. Purity (SDS-PAGE): Single major band
5. Molecular Weight (SDS-PAGE): 25.8 ± 2.6 kDa.

## Unit Definition

One unit (U) is defined as the amount of enzyme required to cleave 95% of 0.5 mg fusion protein (in 25mM Tris-HCl, pH 8.0) at 25℃ within 12–16 hours.

## Protocol

1. **Digestion at 25℃:**

   Based on the unit definition, a typical digestion reaction is as follows:

   In a 25mM Tris-HCl (pH 8.0) system:

   Fusion protein: 0.1–1mg/mL (total protein: 50–100μg)

   Recombinant enterokinase: 0.1–0.2 U

   Incubate at 25℃ overnight (16–24 hours for complete digestion). Analyze digestion efficiency.

2. **Digestion at 4℃ (Low Temperature)**

   fficient digestion can be achieved at 4℃, but requires extended incubation time: 48–64 hours, or increased enzyme amount: 2–3 times the standard dosage.

3. **Digestion Optimization and Scale-Up**

   Optimization: Adjust buffer pH, fusion protein concentration, enzyme-to-substrate ratio, and incubation time to identify optimal conditions for your specific protein. Verify results by SDS-PAGE.

Scale-Up: Scale the reaction proportionally using the optimized conditions. Confirm digestion efficiency by SDS-PAGE.

Removal of Recombinant Enterokinase: Remove enterokinase using trypsin inhibitor affinity chromatography.

Note: Digestion at 37℃ is not recommended, as it may lead to non-specific cleavage.

## Factors Affecting Enterokinase Activity

The enzyme digestion effect is affected in>200mM imidazole, or>200mM NaCl, or>5% glycerol. You can refer to the following recommended methods for enzyme digestion:

1. For optimal results, dialyze your sample into 25mM Tris-HCl (pH 8.0) before digestion.
2. If dialysis is not feasible, dilute the sample to Imidazole < 100mM, Imidazole < 100mM, Glycerol < 5%, maintain the standard enzyme-to-protein ratio (1U per 500μg protein).
3. If inhibitory components cannot be removed, increase the enzyme amount or extend the incubation time to achieve satisfactory digestion.