Enterokinase Cleavage Enzyme

Cat. No. G699

Store at -20°C.

Product Description

abm's **Enterokinase Cleavage Enzyme** is the catalytic subunit of the native porcine holoenzyme. It is highly active and specific for cleaving fusion proteins with the recognition sequence, Asp-Asp-Asp-Asp-Lys (DDDDK), in the inter-domain linker. Unlike other site-specific proteases that cut within the recognition sequences leaving extra amino acids in the cleaved peptide products, the Enterokinase cleaves after the C-terminal end of the lysine residue and the fragment produced from the cleavage reaction does not inherent any residues from the DDDDK recognition sequence. Therefore, the application can be extremely advantageous for producing a 100% native protein sequence, and structure from recombinant fusion protein, which has the desired product immediately after the enterokinase recognition sequence, DDDDK. Owing to the presence of a His tag at the N-terminus, **abm**'s Enterokinase can be easily removed after the cleavage reaction by affinity chromatography with Ni-IDA Agarose Beads (**abm** Cat. No. G250).

In addition, DDDDK is part of the octapeptide FLAG tag (DYKDDDDK), which can be utilized as a fusion tag for recognition by antibody, and detection of fusion protein expression with Western blot analysis, as well as for purification of the fusion protein by Anti-FLAG affinity chromatography. This array of applications makes Enterokinase an ideal tool in the research involving the study of protein structure and function, and protein production where native protein structures and sequences are desired.

Product Component	Quantity	Part No.
Enterokinase Cleavage Enzyme	100 µl (1 U/µl)	G699

Product Applications

- Cleavage of tags from recombinant fusion proteins containing an Enterokinase recognition site.
- One step affinity removal of his-tagged Enterokinase after cleavage.
- No residues left from recognition sequence after cleavage.

Protocol

To determine the optimal cleavage condition of the target protein by Enterokinase Cleavage Enzyme, a sufficient amount of the target protein (i.e.,150 µg with a concentration of at least 1 mg/ml) is needed at the beginning. Provided that a sufficient amount of protein substrate is present in the reaction (i.e., 0.4 mg/ml), the parameters that influence the cleavage efficiency include 1) final concentration of Enterokinase in the reaction, 2) reaction temperature, 3) reaction time, and 4) composition of buffer in the reaction. These parameters vary with different target proteins because each target protein presents the DDDDK cleavage sequence differently in their three-dimensional structures in solution. The following recipe is an example of a grid search, composed of a number of small-scale digestion reactions. It is designed to estimate the optimal Enterokinase concentration in the reaction, and at the same time find out the length of time needed for the reaction to finish.

10X Recommended Reaction Buffer Components (Not provided)
200 mM Tris-HCl, 500 mM NaCl, 20 mM $CaCl_2$, pH 8.0.

1. Dilute Enterokinase in Enzyme Storage Buffer (*20 mM Tris-HCl (pH 8), 200 mM NaCl, 2 mM $CaCl_2$, and 50% (v/v) Glycerol*) to concentrations varying from 0.01, 0.04, 0.1, 0.4, and 1 U/µl.
2. Add the following components to well-labeled tubes:

Product Component	Volume
Fusion Protein	Variable
Diluted Enterokinase Cleavage Enzyme	5 µl
10X Recommended Reaction Buffer	5 µl
Nuclease-Free H_2O	up to 50 µl

3. Collect all components by a brief centrifugation.
4. Incubate the reaction at 25°C and remove aliquots for analysis at 2, 9 and 24 hours, and prepare aliquots for analysis by SDS-PAGE
5. Determine the optimal length of time for cleavage by analyzing the amount of cleaved and uncleaved protein at each time point. Use this information to optimize the protein cleavage.

General Notes

- We recommend setting up a Negative Control (no Enterokinase) in which 5 µl of Enzyme Storage Buffer is used in place of Enterokinase..
- Store all components at -20°C. Avoid repeated freeze-thaw cycles of all components to retain maximum performance. All components are stable for one year from the date of shipping when stored and handled properly.

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