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Enteropeptidase/Enterokinase Cleavage Kit

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(Catalog # K760-100; 100 reactions; Store kit at -20°C)

I. Introduction:

Enteropeptidase (Enterokinase, EC **3.4.21.9**) is a serine protease involved in activation of trypsinogen to trypsin, which in turn results in the activation of various digestive enzymes. It recognizes a highly specific amino acid sequence '**DDDDK**' and cleaves after the lysine residue (**K**). The high specific activity of Enteropeptidase has been utilized in cleaving a variety of native or fusion proteins containing the above recognition motif. Biovision's Enteropeptidase Cleavage Kit contains highly active light chain fragment of human Enteropeptidase. Our pure enzyme is an excellent tool to obtain a wild type protein sequence from a fusion protein, containing Enteropeptidase recognition sequence (**DDDDK**). This Enteropeptidase cleavage kit is sufficient for cleaving at least 10 mg of target protein. The residual enteropeptidase left in the reaction mix will not interfere with most of the downstream applications. Following cleavage of the target protein, Enteropeptidase can be removed using BioVision Hi-Bind Ni-QR Agarose beads.

II. Application:

Efficiently removes tags from recombinant fusion proteins containing accessible Enteropeptidase-specific recognition sequence.

III. Kit Contents:

Components	K760-100	Cap Code	Part Number
Enteropeptidase Cleavage Buffer	20 ml	WM	K760-100-1
Human Enteropeptidase	1 vial	Green	K760-100-2
Cleavage Control Protein (hPRL-Trx) (50 µg)	1 vial	Blue	K760-100-3

IV. User Supplied Reagents and Equipment:

- Sterile Eppendorf tubes or Falcon tubes.
- 50% glycerol.

V. Storage and Handling:

Store kit at –20°C. Warm Enteropeptidase Cleavage Buffer to room temperature before use. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

VI. Reagent Preparation and Storage Conditions:

- **Enteropeptidase:** Supplied at high activity concentration in 50% glycerol. It can be diluted 10-fold in 50% glycerol (not supplied). Aliquot & keep at -80°C for long term storage. Avoid repeated freeze/thaw. Use within two months.
- **Enteropeptidase working solution:** Dilute Enteropeptidase 100-fold in the Enteropeptidase Cleavage Buffer and keep on ice. Prepare as needed. Do not store diluted enteropeptidase solutions.
- **Cleavage Control Protein**: Reconstitute with 50 µl of Enteropeptidase Cleavage Buffer to obtain 1 µg/µl Control Protein solution.

VII. Enteropeptidase Cleavage Protocol:

a. Target Protein: Dilute your target fusion protein to final concentration of 1-5 mg/ml with appropriate volume of Enteropeptidase Cleavage Buffer.

b. Reaction Mix: In a sterile eppendorf tube, mix enough of the following reagents for the number of cleavage reactions to be performed:

	Target Protein Mix (50 µg)	Cleavage Control Protein Mix (10 µg)
Target Protein	50 µl	-
Control Protein	-	10 µl
Enteropeptidase working solution:	5 µl	2 µl

Mix gently by pipetting up and down (do not vortex) and gently agitate/rotate at 37 °C for 4-8 hrs. Aliquot ~10 µl (10 µg) from the target protein reaction mixture at regular time intervals and freeze at -20 °C. After the digestion, collect all the samples and analyze by SDS-PAGE. For the Cleavage Control Protein, run 5 µg of undigested Cleavage Control Protein along with the digested Cleavage Control Protein mix (5 µg) after digestion.

Note: Successful cleavage with the Enteropeptidase is dependent upon properties of the fusion protein allowing for easier exposure of the enzyme recognition sequence. In order to find the optimum cleavage conditions (time and the amount of enzyme used), it is recommended to run preliminary digestion reactions at a small scale. *A recommended starting point is 1-5 µl of stock enzyme per milligram of target protein*. The enzyme stock solution can be further diluted with the Enterokinase Cleavage Buffer to obtain 1/10, 1/100 and 1/1000 dilutions etc. Once optimum cleavage conditions are obtained, the reaction can be scaled up to digest the entire amount of the target protein.

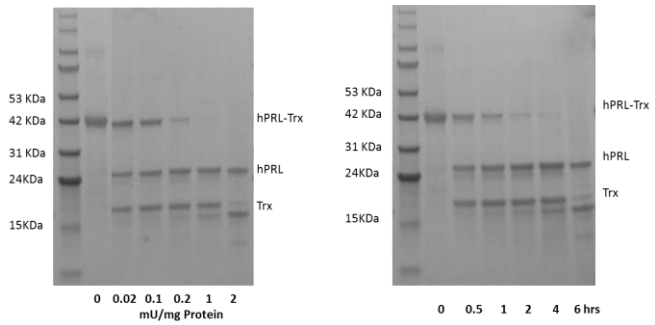

Figure: SDS-PAGE analyses of hPRL-Trx (42.3 kDa). hPRL-Trx (50 µg) was digested into its individual protein fragments hPRL (25.3 kDa) and Trx (17 kDa), .
A: hPRL-Trx digestion using various amounts of Enteropeptidase (0.02-2 mU) per milligram of hPRL–trx at 37 °C for 6 hrs.
B: hPRL-Trx digestion at different time points at RT using 2 mU of Enteropeptidase per milligram of hPRL–trx at 37 °C

Enteropeptidase is determined by using Enteropeptidase/Enterokinase Activity Fluorometric Assay Kit (BioVision, K758). One unit of Enteropeptidase is the amount of enzyme that generates 1.0 µmol of AFC per min at 37 °C.

VI. RELATED PRODUCTS:

Enteropeptidase Activity Assay Kit
Enteropeptidase Inhibitor Screening Kit
Asparaginase Activity Assay Kit
Granzyme B Activity Assay Kit
Granzyme B Inhibitor Screening Kit
Lipase Activity Assay Kit
Lipase Activity Assay Kit II
Lipase Activity Assay Kit III
Protease Activity Assay Kit
Endothelial lipase antibody
Endothelial lipase blocking peptide
Protease Inhibitor Cocktail
EZBlock™ Protease Inhibitor Cocktail
EZBlock™ Protease Inhibitor Cocktail, EDTA-Free

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