ab207001 Enterokinase cleavage kit

Instructions for use:

For efficiently removing tags from recombinant fusion proteins containing accessible enteropeptidase-specific recognition sequence.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 6b, Last Updated 08 December 2023

Table of Contents

INTRODUCTION 1

1. BACKGROUND 1

2. ASSAY SUMMARY 2

GENERAL INFORMATION 3

3. PRECAUTIONS 3

4. STORAGE AND STABILITY 3

5. LIMITATIONS 3

6. MATERIALS SUPPLIED 4

7. MATERIALS REQUIRED, NOT SUPPLIED 4

8. TECHNICAL HINTS 4

ASSAY PREPARATION 5

9. REAGENT PREPARATION 5

ASSAY PROCEDURE 6

10. ASSAY PROCEDURE 6

DATA ANALYSIS 7

11. TYPICAL DATA 7

RESOURCES 8

12. QUICK ASSAY PROCEDURE 8

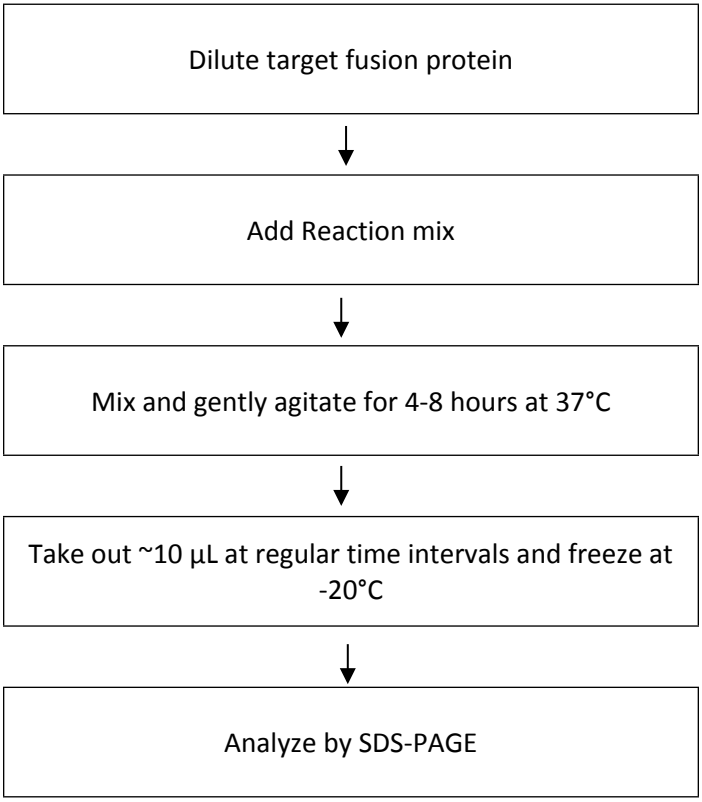
13. NOTES 9

1. BACKGROUND

Abcam's Enterokinase cleavage kit (ab207001) efficiently remove tags from recombinant fusion proteins containing accessible enteropeptidase-specific recognition sequence.

Enteropeptidase (enterokinase, EC 3.4.21.9) is a serine protease involved in activation of trypsinogen to trypsin. It recognizes a highly specific amino acid sequence 'DDDDK' and cleaves after the lysine (K) residue. The high specific activity of enteropeptidase is also used to cleave native and fusion proteins containing this recognition motif, and is often used to remove the tag from a recombinant protein. Abcam's Enterokinase cleavage kit contains a 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 that contains the enteropeptidase recognition sequence. This kit is sufficient for cleaving at least 5 mg of target protein. The residual enteropeptidase left in the reaction mix will not interfere with most experiments that the target protein will be used in. Following cleavage of the target protein, the enteropeptidase may be removed using nickel or cobalt beads if required. However, this is only possible if the target protein contains a histidine residue. If no histidine residue is present, enteropeptidase cannot be removed.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 6 and 9.

5. LIMITATIONS

- Kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Enteropeptidase Assay Buffer/Enteropeptidase Cleavage Buffer	20 mL	-20°C	-20°C
Human Enteropeptidase II/Human Enteropeptidase	1 vial	-20°C	-80°C
Cleavage Control Protein/Cleavage Control Protein (hPRL-Trx) (50 µg)	1 vial	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Sterile microcentrifuge tubes or disposable 15 mL and 50 mL tubes
- 50% glycerol

8. TECHNICAL HINTS

- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening

9.1. **Enteropeptidase Assay Buffer/Enteropeptidase Cleavage Buffer:**

Ready to use. Warm to room temperature before use.

9.2. **Human Enteropeptidase II/Human 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.

To prepare a Enteropeptidase working solution, dilute the Enteropeptidase 100-fold in the Enteropeptidase Assay Buffer/Enteropeptidase Cleavage Buffer and keep on ice. Prepare as needed. Do not store diluted enteropeptidase solutions.

9.3. **Cleavage Control Protein:**

Reconstitute with 50 µL of Enteropeptidase Assay Buffer/Enteropeptidase Cleavage Buffer to obtain 1 µg/µL Control Protein solution. Once reconstituted, aliquot and store at -20°C for up to 6 months. Avoid repeated freeze/thaw.

10. ASSAY PROCEDURE

10.1. Dilute the target fusion protein to a final concentration of 1-5 mg/mL with appropriate volume of Enteropeptidase Assay Buffer/Enteropeptidase Cleavage Buffer.

10.2. Use 1 sterile microcentrifuge tube per cleavage reaction. Add the following reagents to each tube. Include 1 cleavage reaction with Cleavage Control Protein.

Component	Target Protein Mix (50 µg)	Cleavage Control Protein Mix (10 µg)
Target Protein	50 µL	N/A
Control Protein	N/A	10 µL
Human Enteropeptidase II/Human Enteropeptidase working solution	5 µL	2 µL

10.3. Mix gently by pipetting up and down (do not vortex).

10.4. Gently agitate at 37°C for 4-8 hours.

10.5. Remove ~10 µL (10 µg) from the target protein reaction mixtures at the start and at regular time intervals after setting up the reaction mixture and freeze at -20°C.

10.6. After the digestion, analyze all the time point samples removed from each reaction by SDS-PAGE. For the Cleavage Control Protein, run 5 µL of undigested Cleavage Control Protein along with the digested Cleavage Control Protein mix (5 µL) 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.*

ASSAY PROCEDURE

11. TYPICAL DATA

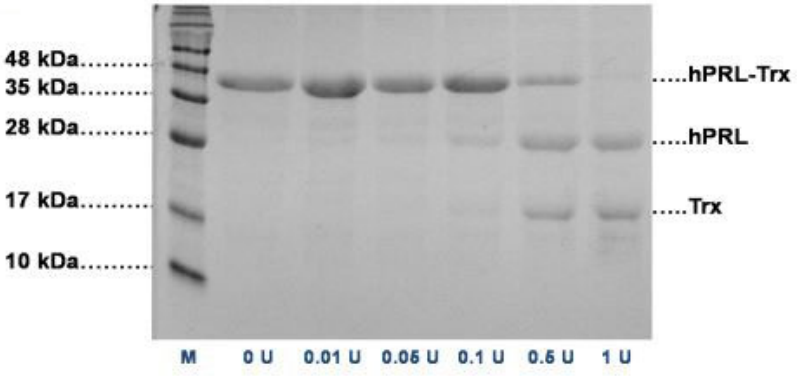


Figure 1: SDS-PAGE analysis of the cleavage of hPRL-Trx using different amounts of enteropeptidase. hPRL-Trx (50 µg) was digested into its individual protein fragments hPRL (25.3 kDa) and Trx (17 kDa) using different amounts (0.01-1 U) of enteropeptidase at room temperature for 20 hours.

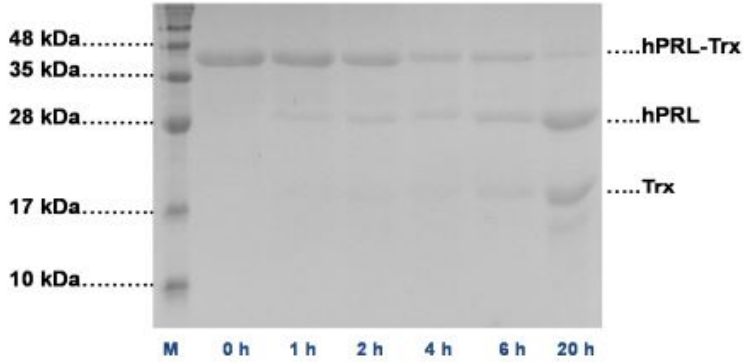


Figure 2: SDS-PAGE analysis of the cleavage of hPRL-Trx by enteropeptidase over time: Analysis of hPRL-Trx digestion at different time points at room temperature using 1 U of enteropeptidase.

12. NOTES

RESOURCES

Technical Support

For all technical or commercial enquiries please go to: www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)