For life science research only.
Not for use in diagnostic procedures.

Enterokinase
Enteropeptidase from calf intestine

Version: 09
Content Version: November 2020

Restriction protease

Cat. No. 11 334 115 001	3 x 30 µg
Cat. No. 11 351 311 001	3 x 250 µg

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Enterokinase	Lyophilized	11 334 115 001	3 vials, 30 µg each
			11 351 311 001	3 vials, 250 µg each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the lyophilizate is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Enterokinase	Store dry at +2 to +8°C.

Storage Conditions (Working Solution)

Store a solution of Enterokinase up to 1 week at +2 to +8°C.

Reconstitution

Dissolve the content of one vial of Enterokinase in double-distilled water to obtain a concentration of 0.25 to 0.3 mg/ml. If necessary, dilute further using the incubation buffer.

1.3. Application

Enterokinase is used for the cleavage of fusion proteins at definite cleavage sites.

- This recognition sequence, however, can also be used as a restriction cleavage site for processing recombinant proteins. For this purpose, the desired protein is fused at the C-terminal of the recognition sequence.
- After purification of the entire fusion protein, the protein or peptide is released by incubation with Enterokinase.
- This release is affected by the adjacent amino acid sequences at the cleavage site as well as by the size of the two fused components and the accessibility of the cleavage site.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Suitable denaturing agents

The following table shows the influence of different denaturing agents on the cleavage rate of Enterokinase. Incubation was carried out in 50 mM Tris buffer, pH 8.0, for 1 hour at +37°C; amount of Enterokinase: 1/40 (w/w) of the fusion protein.

When using urea, add 20 mM methylamine.

To achieve a suitable concentration of the denaturing agent in the reaction mixture, the protein must be correspondingly diluted with incubation buffer.

Denaturant	Concentrations tested	Inhibition of cleavage
Urea + methylamine	0.1 – 3 M	None
SDS	0.01 – 1%	Inhibitory at 0.01%
Hydrogenated Triton X-100	0.01 – 1%	None
Tween 80	0.01 – 1%	None
Acetonitrile	5%, 10%	None

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

2.2. Protocols

Enterokinase cleavage requirements

- Cleavage is carried out at an enzyme/substrate ratio (w/w) of 1/20 to 1/200 for 1 to 24 hours. Suitable conditions for each fusion protein are determined by performing pilot experiments.
- The optimal pH for the reaction is between pH 7.0 to 8.0, however the enzyme can be used within pH 6.0 to 8.5.
- Suitable buffers include 50 mM Tris or 50 mM MES. In phosphate buffer, the activity is significantly reduced.
- Higher concentrations of salts, such as NaCl, inhibit the reaction.
- Addition of $CaCl_2$ to the incubation mixture does not increase the reaction rate.
- Insoluble aggregates of fusion proteins are cleaved extremely slowly or not at all.
- Denaturing of the fusion protein might be necessary to increase the accessibility of the cleavage site.

Pilot experiments

1. Adjust the concentration of the fusion protein to 0.3 to 1 mg/ml and a pH of 7.0 to 8.0.
   – If the fusion protein is dissolved in phosphate buffer or in case of high ionic strength, exchange the incubation buffer either by dialysis or gel filtration.

2. Prepare a solution of Enterokinase with double-distilled water, see section, **Reconstitution**.
   The content of one vial can be used for several simultaneous digests. To repeat the digest, use a new vial to ensure utmost reproducibility and to avoid contamination.

3. Use a small portion of your fusion protein.
   – Incubate 25 µg fusion protein (25 µl with 1 mg/ml) with 0.6 µg Enterokinase (2 µg with 0.3 mg/ml).

4. Remove 5 µg samples of the reaction mixture after 1, 3, 6, and 24 hours.

5. Add 5 µl 2x-concentrated SDS-PAGE sample buffer.

6. Boil for 5 minutes and store at −15 to −25°C until SDS-PAGE is performed.

7. Perform a control incubation without Enterokinase to detect a possible nonspecific cleavage by autolysis or by proteolytic contaminations of the fusion protein.

8. To optimize the amount of Enterokinase necessary for 100% cleavage, perform a similar experiment with variations of the enzyme concentration and 3 hours incubation time.

9. Analyze the samples of the experiments, the corresponding control incubations, and a sample of uncut fusion protein on a SDS-PAGE gel (Fig. 1).

10. Incubate the main portion of the fusion protein using the optimized conditions obtained from the pilot experiments.
    – Check for full cleavage by SDS-PAGE and use as reference, a sample of uncut fusion protein.

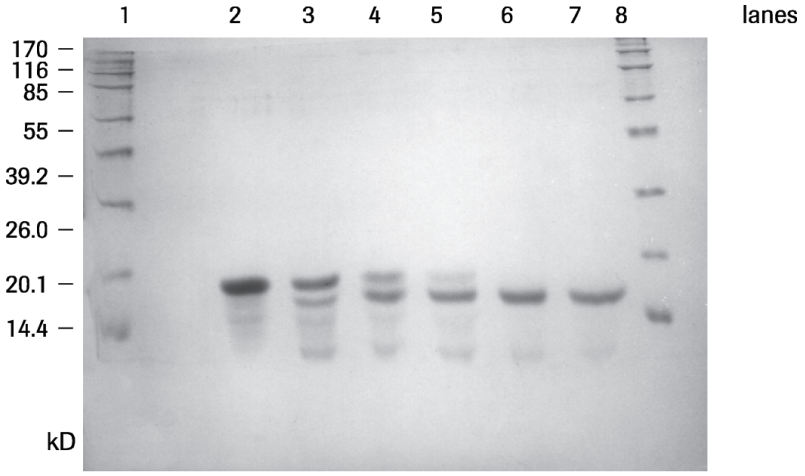

Fig. 1: Cleavage of a fusion protein with Enterokinase. Incubation was carried out in 50 mM Tris buffer, pH 8.0, as described in the pilot experiments.
Lanes 1, 8: Calibration proteins, Combitek.
Lane 2: Uncut fusion protein.
Lanes 3, 4, 5, 6, 7: Incubations of the fusion protein with Enterokinase for 1, 3, 6, 20, and 32 hours, respectively.

2.3. Parameters

EC-Number

Enteropeptidase, EC 3.4.21.9

Molecular Weight

150 kDa

Purity

The restriction protease Enterokinase does not contain any additional protein, such as bovine serum albumin used as a stabilizer. It is present in a highly purified form. Purity control by SDS polyacrylamide gel electrophoresis (SDS-PAGE) ensures a constant quality between lots. The composition of the lyophilizate does not influence the cleavage of the fusion proteins.

Specificity

Enterokinase is a serine protease that recognizes the following amino acid sequence with a high specificity.
-Asp-Asp-Asp-Asp-Lys-↓-X-

- The enterokinase activates its natural substrate trypsinogen and releases trypsin by cleavage at the C-terminal end of this sequence.
- The aspartic acid residues can be partially substituted by glutamic acid.

3. Additional Information on this Product

3.1. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Trademarks

All product names and trademarks are the property of their respective owners.

4.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

1120.11352091001 ⓔ

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany