

Enterokinase

Restriction protease
From calf intestine
Enteropeptidase, E.C. 3.4.21.9

Cat. No. 1 334 115 3 × 30 µg
Cat. No. 1 351 311 3 × 250 µg

Version 3, October 1999

Store at 2-8° C

Product description

Commercial availability

Lyophilized.

Specificity

Enterokinase is a serine protease that recognizes the amino acid sequence

-Asp-Asp-Asp-Asp-Lys-I-X-

with a high specificity. The enterokinase activates its natural substrate trypsinogen and releases trypsin by cleavage at the C-terminal end of this sequence (1). The aspartic acid residues can be substituted by glutamic acid (2).

Application

This recognition sequence, however, can also be used as a restriction cleavage site for processing recombinant proteins (3). 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.

A constant quality is guaranteed by testing each lot with a recombinant fusion protein. The release of the desired protein or peptide component from a fusion protein is affected by the adjacent amino acid sequences at the cleavage site as well as by the size of the two fused components and by the accessibility of the cleavage site (2).

Purity

The restriction protease enterokinase does not contain any further protein like bovine serum albumin as stabilizer. It is present in a highly purified form: Purity control by SDS polyacrylamide gel electrophoresis (SDS-PAGE) ensures a constant quality from lot to lot. The composition of the lyophilisate does not influence the cleavage of the fusion proteins.

Note

The content of one vial may be used for several simultaneous digests. In order to repeat the digest a new vial should be taken. Thereby the utmost reproducibility can be guaranteed and contamination will be avoided.

Stability

stable at 2-8° C, stored dry. A solution of enterokinase, stored at 2-8° C, can be used up to one week.

Procedure

Enterokinase cleavage is carried out at an enzyme/substrate ratio (w/w) of 1/20-1/200 for 1-24 h. For each fusion protein pilot experiments should be done to find out suitable conditions. The optimal pH for the reaction is between 7.0-8.0, but the enzyme can be used within pH 6.0-8.5. Suitable buffers are, 50 mM Tris* or 50 mM Mes*. In phosphate buffer the activity is significantly reduced. Higher concentrations of salts, e.g. NaCl, inhibit the reaction. Addition of CaCl₂ to the incubation mixture does not increase the reaction rate (4). 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. Suitable denaturing agents are listed in the table. On application of urea it is recommended to also add 20 mM methylamine. In order to achieve a suitable concentration of the denaturing agent in the reaction mixture, the protein has to be correspondingly diluted with incubation buffer.

1. Adjust the concentration of the fusion protein to 0.3-1 mg/ml and a pH between 7.0-8.0. If the fusion protein is dissolved in phosphate buffer or in case of high ionic strength, it is necessary to exchange the incubation buffer either by dialysis or gel filtration.
2. Dissolve the content of a vial of enterokinase in redist. water to obtain a concentration of 0.25-0.3 mg/ml. If necessary, a further dilution should be done using the incubation buffer.
3. Pilot experiments:
Carry out the pilot experiments with a small portion of your fusion protein.
Incubate 25 µg fusion protein (25 µl with 1mg/ml) with 0.6 µg enterokinase (2 µg with 0.3 mg/ml). Take 5 µg samples of the reaction mixture after 1, 3, 6 and 24 h, add 5 µl 2 × concentrated SDS-PAGE sample buffer, boil for 5 min and store at -15 to -25°C until SDS-PAGE is performed. To detect a possible unspecific cleavage either by autolysis or by proteolytic contaminations of the fusion protein a control incubation without enterokinase is recommended.
To optimize the amount of enterokinase necessary for 100% cleavage do a similar experiment with variations of the enzyme concentration and 3 h incubation time.
Analyse the samples of an experiments, the corresponding control incubations and a sample of uncut fusion protein on a SDS-PAGE gel (5).

4. Incubate the main portion of the fusion protein using the optimized conditions deduced from the pilot experiments. Check for complete cleavage by SDS-PAGE and use as reference a sample of uncut fusion protein.

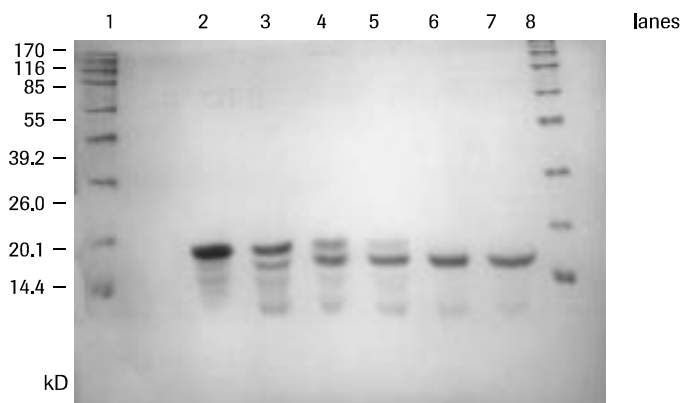


Fig.: Cleavage of a fusion protein with enterokinase. Incubation was carried out in 50 mM Tris buffer, pH 8.0, as described for the pilot experiments in the procedure. Lanes 1, 8: calibration proteins, combihek[®]; lane 2: uncut fusion protein; lanes 3, 4, 5, 6, 7: incubations of the fusion protein with enterokinase for 1, 3, 6, 20 and 32 h respectively.

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

Tab.: Influence of different denaturing agents on the cleavage rate of enterokinase. Incubation was carried out according to the procedure in 50 mM Tris buffer, pH 8.0, for 1 h at 37°C; amount of enterokinase: 1/40 (w/w) of the fusion protein.

References

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- Fonseca, P. & Light, A. (1983) *J. Biol. Chem.* **258**, 14516-14520.
- Laemmli, U. K. (1970) *Nature* **227**, 680-685.

* available from Roche Molecular Biochemicals

¹⁾ Triton X-100 is a trademark of Rohm & Haas, Philadelphia/USA.

²⁾ Tween 80 is a trademark of ICI Americas Inc., Wilmington/USA.

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