3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Enterokinase
from bovine intestine

Catalog Number **E5144**
Storage Temperature –20 °C

CAS RN 9014-74-8
EC 3.4.21.9
Synonym: Enteropeptidase

Product Description
Enterokinase is a highly specific serine protease used for the removal of the FLAG® peptide from fusion proteins. It is supplied as a Na^+ Cl^- form, lyophilized from deionized water.

Recognition Peptide:
LYS-X of FLAG peptide
N-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-X*-C

*Peptides are resistant to cleavage if proline occupies position X.

Bovine enterokinase, a glycoprotein containing 35% carbohydrate, is a 150 kDa heterodimer consisting of 115 kDa and 35 kDa subunits. Two disulfide bridges link the light and heavy chains.

Bovine enterokinase has been shown to be inhibited by soybean trypsin inhibitor.

Unit definition: One unit is that amount of enterokinase which results in >95% cleavage of 1 µg of purified FLAG•BAP fusion protein in 18 hours at 37 °C.

One FLAG•BAP unit is equal to 10× the activity of a standard trypsinogen unit.[1]

Reagents
The enterokinase is shipped with a vial of enterokinase buffer, 10 mM Tris HCl, pH 8.0, with 10 mM $CaCl_2$ (Catalog Number E7645). The enterokinase buffer is used as both reaction buffer and as storage buffer.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store the enzyme product at –20 °C. Resuspend the enzyme immediately prior to use in the enterokinase buffer to a working concentration of 1 unit/µl. The resuspended enzyme is stable for 1 month when stored at –20 °C. For longer periods store aliquots of enzyme at –70 °C. Store the enterokinase buffer at 2–8 °C.

Procedure
Optimal results have been obtained with FLAG fusion protein concentrations at ~250 ng/ml. The pH of the FLAG fusion protein solution should be adjusted to a pH between 7.4 and 8.0.

Add ~5.0 units of enterokinase per µg of FLAG fusion protein. Digestion should be carried out in the enterokinase buffer. Mix and incubate the digestion mixture at 37 °C overnight.

Removal of the FLAG peptide can be determined by a dot blot on nitrocellulose using ANTI-FLAG® M2 antibody (Catalog Number F3165) or ANTI-FLAG M1 antibody (Catalog Number F3040). The free peptide does not bind to nitrocellulose; whereas, the FLAG fusion protein does. Free FLAG peptide will bind to PVDF membranes, so PVDF is not recommended. FLAG peptide removal may also be confirmed by SDS-PAGE analysis, since the native protein is 1 kDa less than the intact FLAG fusion protein. The free FLAG peptide can also be removed from the fusion protein by gel filtration, dialysis, or ultra-filtration. Enterokinase can be removed by gel filtration or affinity chromatography depending on the nature of the fusion protein.

References
Liepnieks, J., and Light, A., J. Biol. Chem., **254**, 1677-1683 (1979).

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