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Product Information

Enterokinase Removal Kit

Catalog Number **PRKE**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Enterokinase (EC 3.4.21.9) is a highly specific mammalian serine protease that recognizes the amino acid sequence $(Asp)_4$-Lys-↓-X. Partial substitution of Asp residues by Glu is possible. The cleavage site $(Gly)_4$-Lys-↓-X is also recognized. These sequences are relatively uncommon in natural proteins. Enterokinase cleaves its natural substrate trypsinogen to form trypsin.[1-3]

Enterokinase is used for site specific cleavage of recombinant fusion proteins containing an accessible enterokinase recognition site for removal of affinity tags.[4] Cleavage is done either before or after fusion protein purification.

The Enterokinase Removal Kit is designed as a research tool for the removal of bovine enterokinase from mixtures containing a fusion protein cleaved by the enzyme. Using the kit, removal of essentially all enterokinase (as judged by antigenic or enzymatic activity assays) is accomplished by binding with immobilized rabbit antibodies to calf intestine enterokinase followed by spin filtration. The protein of interest remains in the filtrate. Enterokinase, its fragments and aggregates as well as some contaminating proteins are depleted from the cleavage mixtures regardless of their enzymatic activity.

100 µl of a 50% suspension (50 µl packed gel) of the anti-enterokinase-agarose will bind 0.2–1 µg of bovine enterokinase or recombinant catalytic subunit. Removal of recombinant bovine enterokinase catalytic subunit may be achieved by this kit.

For applications demanding more stringent removal of enterokinase, repeated capture steps are recommended. Inactivation of any residual activity may be carried out with enterokinase inhibitors. The suitability for quantitative removal of enterokinase derived from non-bovine sources must be determined by the user.

Components

Anti-Enterokinase-Agarose Conjugate 1.5 ml
(Catalog Number A4838)
3 ml of a 50% suspension in 0.01 M phosphate buffered saline containing 15 mM sodium azide

20× Wash Buffer 4 ml
(Catalog Number B3803)
0.4 M Tris-HCl, pH 7.4, containing 1 M NaCl and 0.04 M $CaCl_2$

Spin Filters 10 each
(Catalog Number S3563)
Pre-assembled

Reagents and Equipment Required but Not Provided.

- Deionized water
- Fusion protein-enterokinase mixture (cleavage reaction mixture)
- Benchtop centrifuge, variable speed
- 1.5 ml microcentrifuge tubes or polypropylene test tubes
- Timer
- Pipettes and tips
- Rocker/Rotator

Precautions and Disclaimer

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

Preparation Instructions

1× Wash Buffer – Dilute required amount of 20× Wash Buffer 20-fold with distilled water.

Storage/Stability

Store all components at 2–8 °C.
Notes: Do not freeze.

Spin filters may be stored at room temperature.

Procedure

The following procedure is recommended for a cleavage mixture of 100 µl containing 0.2–1 µg of enzyme. For different volumes, calculate accordingly (see Note 1).

All steps are carried out at room temperature.

1. Dilute 20× Wash Buffer 20-fold with deionized water. 1.1 ml of 1× Wash Buffer is needed for 50 µl of packed gel.

2. Resuspend the anti-enterokinase–agarose conjugate by inversion until fully resuspended. Transfer 100 µl of suspension to a microcentrifuge tube and wash with 500 µl (10 bed volumes) of 1× Wash Buffer by centrifugation at 1,000 × *g* for 2 minutes. Remove the supernatant and repeat the wash step. Resuspend the agarose in 50 µl (1 bed volume) of 1× Wash Buffer.

3. Transfer the agarose suspension to the cup of the pre-assembled spin filter.

4. Add the cleavage reaction mixture and mix gently on a rocker/rotator for 45 minutes. Do not vortex.

5. Centrifuge at 1,000 × *g* for 2 minutes to collect the enterokinase-depleted filtrate.

6. Analyze the filtrate by SDS-PAGE or activity assay to ensure full recovery of the protein of interest.

Notes:

1. For removal of enterokinase from total volumes in excess of 0.3 ml, the spin filters cannot be used. Use microcentrifuge tubes/polypropylene test tubes. Following centrifugation as in step 5, collect the supernatant by aspiration.

2. Discard used spin filters. Do not attempt to regenerate the anti-enterokinase-agarose conjugate.

3. A cleavage/capture buffer of choice may be substituted for the 1× Wash Buffer.

4. Use of buffers with pH >8.5 is not recommended.

References

1. Kell, B., The Enzymes, 3rd Ed., Vol. 3, p. 249, Boyer, P.D., ed. (Academic Press, New York: 1971).
2. Andersson, L.E. *et al*., Biochemistry, **16**, 3354 (1977).
3. La Vallie, E.R., *et al*., J. Biol. Chem., **268**, 23311 (1993).
4. La Vallie, E.R., and McCoy, J.M., "Enzymatic Cleavage of Fusion Proteins with Enterokinase" in Current Protocols in Molecular Biology, Vol. 2, Supplement 28, Ausubel, F.M., ed., (John Wiley & Sons, New York: 1994) p.16.4.10-16.4.11.

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