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## I DESCRIPTION

Enterokinase (EK) is a specific protease that cleaves a protein after lysine residue at its recognition site: Asp-Asp-Asp-Asp-Lys. Enterokinase will not work if the recognition site is followed by proline. If a fusion tag is placed at the N-terminus with an enterokinase site, enterokinase will be able to remove the fusion tag and to generate the protein exactly as you need without adding any unwanted residues. GenScript **Enterokinase, Light Chain, Porcine** is a highly purified recombinant porcine enterokinase (Light Chain) expressed from *P. Pastoris* GS115.

## II COMPONENTS

100 U Recombinant Enterokinase, 2 U/μl, 50 μl in all.

2 ml 1X EK Dil./Stor. Buffer

1 ml 10X EK cleavage buffer

10 μg Cleavage control protein, lyophilized powder on the bottom of the tube. (The molecular weight of control protein is 26,000 Da before cleavage. After cleavage, molecular weights of the two fragments are 17,000 Da and 9,000 Da.)

## III MOLECULAR WEIGHT

Theoretical MW: 21,880 Da; The apparent MW on SDS-PAGE: about 40,000 Da.

## IV FORMULATION

GenScript Enterokinase, Light Chain, Porcine has been formulated using a proprietary technology, and the enzyme can be shipped at room temperature or stand at 37°C for 7 days without losing any activity.

## V UNIT DEFINITION

One unit is defined as the amount of enzyme needed to cleave 50 μg of fusion protein in 16 hours to 95% completion at 22°C in a buffer containing 25 mM Tris-HCl, pH 8.0.



## VI GENERAL PROTOCOL

### Small scale optimization

Preliminary small scale digestion is recommended in order to find an optimal cleavage condition and enzyme: target protein ratio. Scale up the reaction using the optimized conditions. In this approach, a constant amount of target protein is added to different amounts of protease. Samples are analyzed after the same incubation time.

1. Make a serial dilution of Enterokinase (2 U/ $\mu$ l) diluted by 1X EK Dil./Stor. Buffer in 8 tubes so that there is 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001 and 0 U enzymes in 8 different tubes respectively.
2. Set up the digestion reactions in the 8 labeled tubes by adding to 1  $\mu$ l of diluted EK to 50  $\mu$ g of fusion protein with 1 mg/ml. EK can work fine in both Tris-HCl and phosphate Buffer between pH 7-8.4. Protein sample can be adjusted pH between 7-8.4 using 10X EK Cleavage Buffer. Incubate the reactions at room temperature (20°C - 22°C) for 16 hours.
3. 10  $\mu$ l from each tube is loaded on a SDS-PAGE to determine the extent of cleavage. (See Fig.1.).
4. Determine the desired ratio of Enterokinase to the target protein ( U:  $\mu$ g ).

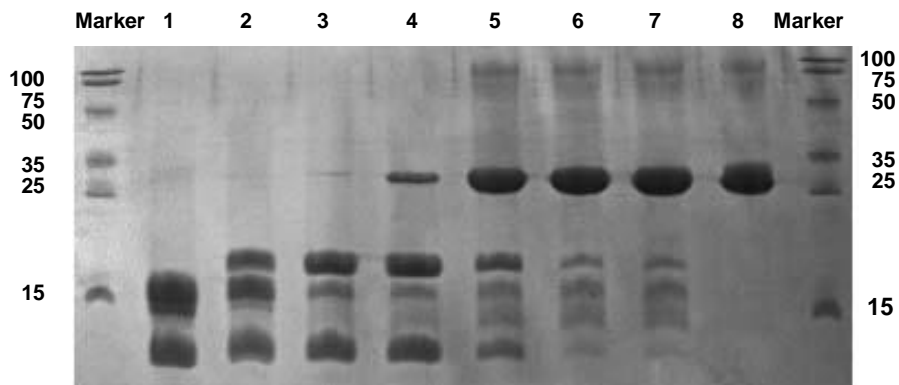


Fig.1. Each reaction contains 50  $\mu$ g of purified IL-8 fusion protein and varying amount of GenScript rPorcine Enterokinase. The reactions were incubated at 22°C for 16 hours and analysed on a coomassie-stained SDS-PAGE (15%). Units of enzyme used per reaction is listed below:

Lane 1. 1 U                      Lane 2. 0.1 U                      Lane 3. 0.01 U                      Lane 4. 0.001 U  
Lane 5. 0.0001 U                      Lane 6. 0.00001 U                      Lane 7. 0.000001 U                      Lane 8. 0 U

5. If the Enterokinase does not work well to your protein sample, you could use Cleavage control protein as a cleavage substrate according to the protocol to confirm the activity of Enterokinase.



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**Scale up**

1. Dialyze the protein sample against 25 mM Tris-HCl, pH 8.0 for overnight at 4°C.
2. Add Enterokinase to target protein at the pre-determined EK : target protein ratio and incubate for 16 hours at 22°C.
3. Analyze samples by SDS-PAGE and Coomassie Blue staining.

**VII STORAGE**

Store at -20°C after receiving.

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