Enterokinase, Light Chain, Porcine **Cat. No. Z01003**

Technical Manual No. 0365 **Version: 08012008**

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 recombinat 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. Prepare 1X Cleavage Buffer by diluting 10X Cleavage Buffer with Sterile Deionized Water. Chill at 4℃ prior to use.
2. Make a serial dilution of Enterokinase (2 U/µl) in 8 tubes so that there is 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001 and 0 U/µl enzymes in 8 different tubes, respectively.
3. Set up the digestion reactions in the 8 labeled tubes by adding to 1 µl of diluted EK to 50 µg of fusion protein with 1 mg/ml. EK can work fine in both Tris-HCl and phosphate buffer between pH 7-8.4. Incubate the reactions at room temperature (20°C - 22°C) for 16 hours.
4. 10 µl from each tube is loaded on a SDS-PAGE to determine the extent of cleavage. (See Fig.1.).
5. Determine the desired ratio of Enterokinase to the target protein (U: µg).

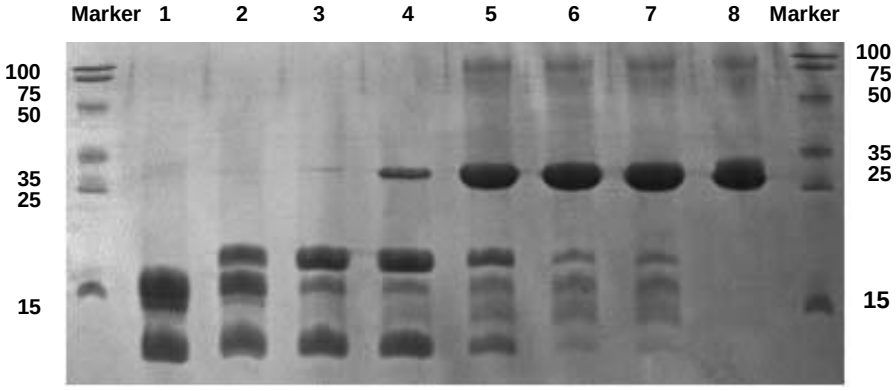

Fig.1. Each reaction contains 50 µg of purified IL-8 fusion protein and varying amount of GenScript rPorcine Enterokinase. The reactions were incubated at 22°C for 16 hours and analysed on a coomassie-stained SDS-PAGE (15%). Units of enzyme used per reaction is listed below:

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

Scale up

1. Prepare 1X Cleavage Buffer by diluting 10X Cleavage Buffer with sterile water. Chill at 4**°C** prior to use.
2. Add Enterokinase to target protein at the pre-determined EK : target protein ratio and incubate at room temperature for 16 hours.
3. Analyze samples by SDS-PAGE and Coomassie Blue staining.

VII STORAGE

Store at -20°C after receiving.

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