Recombinant Enterokinase

Cat.NO.: SGZ 01003

PRICE: EUR64.00/100U

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. ShineGene Enterokinase, Light Chain, Porcine is a highly purified recombinant porcine enterokinase (Light Chain) expressed from *P. Pastoris* GS115.

COMPONENTS

- 100 U  Recombinant Enterokinase, 2 U/μl, 50 μl in all.
- 2 ml   1X EK Dilution/Storage Buffer
- 1 ml   10X EK cleavage buffer
- 10 μg  Cleavage control protein, lyophilized powder on the bottom of the tube.

MOLECULAR WEIGHT

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

FORMULATION

ShineGene 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.

UNIT DEFINITION

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

GENERAL PROTOCOL

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.

1. 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 enzymes in 8 different tubes, respectively. 2. Set up the digestion reactions in the 8 labeled tubes with 50 μg of protein in a total volume of 50 μl per tube.
3. 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.).
Fig.1. Each reaction contains 50 μg of purified IL-8 fusion protein and varying amount of ShineGene 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:

<table>
<thead>
<tr>
<th>Lane</th>
<th>Units</th>
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<tbody>
<tr>
<td>1</td>
<td>1 U</td>
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<tr>
<td>2</td>
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<tr>
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<tr>
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<td>7</td>
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<td>8</td>
<td>0 U</td>
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</tbody>
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STORAGE

Store at -20°C after receiving.

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