Stemimmune LLC
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Recombinant PreScission Protease

Cat# EPE-P-500，EPE-P-1000，EPE-P-5000，EPE-P-10000

Source: E. coli derived

Purity: >90% by SDS-PAGE

Endotoxin Level: <1.0 EU/ug

Molecular Weight: 24kDa

Shipping: The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage

Store recombinant SUMO protease at -80°C for long term or at -20°C for < 6 months. Store 10X PreScission buffer at -20°C. Avoid repeated freeze-thaw cycles.

Description

PreScission Protease is a recombinant human rhinovirus (HRV 3C). PreScission Protease is a cysteine protease that recognizes the eight-amino-acid sequence LEVLFQGP and cleaves between Q and G with high specificity. The protease can be used to cleave affinity tags from recombinant fusion proteins. The optimal temperature for cleavage is 30°C, however, the enzyme is active over wide ranges of temperature and pH (pH 7.0zyme i Following digestion, PreScission Protease is easily removed from the cleavage reaction by affinity chromatography using the polyhistidine tag at the N-terminus of the protease. PreScission Protease is purified from E. coli by affinity chromatography using the polyhistidine tag.

Components

1. PreScission Protease(10U/ul)
   25 mM Tris-HCl, pH 8.0
   150mM NaCl
   5mM DTT
   1mM EDTA
   50% (v/v) glycerol
2. 10X PreScission reaction buffer
   500mM Tris-HCl, pH 7.0
   1.5M NaCl
   10mM DTT
   10mM EDTA

Unit Definition

One unit of PreScission Protease cleaves 90% of 100ug control substrate in 16 h at 4°C.

Unit Assay Conditions

The PreScission Protease assay is performed in 1X PreScission reaction buffer with 1 unit enzyme and 100ug control substrate at 4°C for 16 hour in a total volume of 100ul.

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Recommended Conditions for Cleavage of a Fusion Protein

1. Add the following to a microcentrifuge tube:

Fusion Protein	100ug
10XPreScission Buffer	10ul
PreScission Protease	2ul(0.5-5)(10 U/ul)
Water to	100ul

2.Incubate at 30°C, 20°C and 4°C respectively. Remove 30ul aliquots at different hours.

3. Add 10ul 4X SDS sample buffer keep samples at -20°C until experiment is complete.

4.Analyze 20ul of sample by SDS-PAGE using a suitable gel.

The percent protein cleavage is determined by analyzing the amount of cleaved products formed and amount of uncleaved protein remaining after digestion. After evaluating the initial results, you may optimize the cleavage reaction for your specific protein by optimizing the amount of SUMO Protease, incubation temperature, or reaction time. We recommend performing digests at room temperature (20 °C) or 4 °C. The efficiency of cleavage may vary due to the sequences around the cleavage site, conformation and the solubility of the target protein. It is recommended to optimize the protease amount, incubation time and temperatures for each fusion protein to obtain an efficient cleavage.

Removal of PreScission Protease after Cleavage

The PreScission Protease contains a polyhistidine tag at the N-terminus. After cleavage of the fusion protein, remove PreScission Protease from the cleavage reaction by affinity chromatography on a nickel chelating resin. Dilute cleavage reaction with the binding buffer may be necessary to decrease of DTT and EDTA to a permitted concentration for nickel chelating resin. The cleaved native protein will be in the flow-through fractions.

References

1.Pilon A, et al (1997) Biotechnol. Prog. 13:374 .

2.Baker RT, et al (1996)Curr. Opin. Biotechnol. 7 :541.

3.Malakhov, MP, et al (2004)J. Struct. Funct. Genomics 5 :75.

4.Mossessova, E. et al (2000) Mol. Cell 5, 865.