Version: 01
Update: 03/22/2023

DATASHEET

SUMO Protease Animal-Free, His

Cat. No.: Z03691

I. Product Introduction

SUMO protease, also known as Ulp, is a protease that specifically removes small ubiquitin-related modifier (SUMO) in any recombinant SUMO fusion protein. Different from other tag removal proteases such as enterokinase (EK) and TEV which recognize a specific amino acid sequence and cut at a specific cleavage site, SUMO protease recognizes the SUMO tertiary structure and cleave it, more specifically and leaving no residual amino acids.

GenScript's SUMO Protease Animal-Free, His, is the recombinant SUMO protease fragment from *Saccharomyces cerevisiae*. This recombinant enzyme is expressed in *E.coli* and purified to obtain high yields of the active enzyme. It is produced under an animal free process, and is suitable for drug and vaccine development, manufacture and other applications. This product is designed with a C-terminal 6x His tag which can be removed after the SUMO cleavage reaction by purification using Ni^{2+} affinity chromatography resin (Cat. No. L00223) or Ni-charged magnetic beads (Cat. No. L00295).

Source	Recombinant SUMO Protease expressed in *E.coli*
Species	*Saccharomyces cerevisiae*
Tag	His Tag
Molecular Weight (MW)	Predicted MW: 27 kDa
Purity	≥ 95% as analyzed by SDS-PAGE
Endotoxin Level	< 0.2 EU/μg of protein by gel clotting method
Biological Activity	10 U/μl Unit definition: One unit of SUMO Protease cleaves ≥ 85% of 2 μg control substrate in 1 h at 30℃
Storage Buffer	25 mM Tris-HCl, 0.1% Igepal (NP-40), 250 mM NaCl, 0.5 mM DTT 50% (v/v) glycerol, pH 8.0
Storage & Stability	Upon receiving, the product remains stable for up to 6 months at -20 °C. This product is stable for up to 1 week at 37 °C. Avoid repeated freeze-thaw cycles by making single-use aliquots before the solution is stored at -20 °C.

II. Reagents Supplied

Components	Size			Storage
SUMO Protease Animal-Free, His	250 U	1000 U	5000 U	-20 °C
10 × SUMO Protease Cleavage Buffer (+Salt)[1]	1.8 mL	1.8 mL	1.8 mL	-20 °C
10 × SUMO Protease Cleavage Buffer (- Salt)[2]	1.8 mL	1.8 mL	1.8 mL	-20 °C

[1] 500 mM Tris-HCl, 2% Igepal (NP-40), 1.5 M NaCl, 10 mM DTT, pH 8.0.

[2] 500 mM Tris-HCl, 2% Igepal (NP-40), 10 mM DTT, pH 8.0.

III. Customized Additional Assays

GenScript can offer this product according to your requirements and specifications, including ELISA-based identity assay, HCD test, HCP test and Bioburden test. To customize this product, please contact **product@genscript.com**.

IV. Protocols

Cleavage Assay Procedures

One unit of GenScript's SUMO Protease Animal-Free, His, efficiently cleaves 2 µg of control fusion protein in 1 hour to 85% completion at 30 °C. SUMO protease is active over a wide range of temperatures (2-30 °C), ionic strengths (0-400 mM NaCl), and pH ranges (6.0-9.0). However, as the cleavage of the desired protein/peptide from a fusion protein is intrinsically affected by factors such as the size of the two fused fragments and the substrate and buffer conditions, the suitable conditions for each target fusion protein need to be determined by performing pilot experiments. It is recommended to optimize the cleavage conditions for optimal cleavage efficiency by testing different enzyme concentrations, incubation times, incubation temperatures, etc.

1. Optimization of cleavage conditions in a small scale experiment

a. Assemble the reaction master mixes for each amount of SUMO Protease in a 1.5 ml centrifuge tube according to the table below (e.g., the 200 µl reaction system can be selected as a starting volume).

Components	Volume
Fusion Protein	20 µg
10 × SUMO Protease Cleavage Buffer(+/- Salt)	20 µl
SUMO Protease (10 U/µl)	0, 1, 3, or 5 µl
Deionized water	Up to 200 µl

b. Mix thoroughly, aliquot the 200 µl reaction master mixes into 20 µL reactions, then incubate at different temperatures of 30 °C, 25 °C, 16 °C, and 4 °C for different periods of time (see the table below).

Temperature	Incubation Time
30 °C	1 hour
25 °C	2 hours

16 °C	4 hours
4 °C	Overnight (~16 hours)

Note: If the protein of interest is not very stable at high temperature, e.g., 30 °C, it's suggested to incubate at 4 °C overnight (around 16 hours) and increase the amount of SUMO Protease appropriately to ensure digestion.

c. After incubation, determine cleavage efficiency of each condition by SDS-PAGE. Mix 10 µl of each sample with 2.5 µl 4 × LDS Sample Buffer (Cat. No. M00676) containing DTT and 7.5 µl water. Denature each sample at 95 °C for 5 minutes and load onto the gel (20 µl). Select the reaction condition with the highest cleavage efficiency for further scaling up for protein production.

2. Scale Up for Protein Production

Scale up the reaction proportionally according to the reaction condition determined by the above process. e.g., for cleavage of 100 µg fusion protein, follow the reaction recommendations as indicated in the table below.

Components	Volume
Fusion Protein	100 µg
SUMO Protease at optimized working concentration	x µl (10 U/µl)
10 × SUMO Protease Cleavage Buffer(+/- Salt)	50 µl
Deionized water	Up to 500 µl

After the reaction, the excised SUMO tag with His tag (Generally, add a His tag after the SUMO Tag when designing the fused protein sequence for further purification) and SUMO Protease with His tag can be removed by Ni^{+} affinity chromatography to obtain a high-purity target protein.