



**Recombinant yeast SUMO Protease
(rScUlp1)**

Catalog #MB9018-100
500 units

Description

SUMO (Small Ubiquitin-like MOdifiers) Protease 1 (Ulp1, UBI-specific protease 1 from *Saccharomyces cerevisiae*) is a highly active cysteine protease. Rather than recognizing a short sequence such as TEV, Thrombin or Enterokinase, SUMO protease specifically recognizes the tertiary structure of the hSUMO1-3 and yeast Smt3. SUMO fusion tag, as an N-terminal fusion partner, has been shown to enhance solubilized expression in the bacterial, yeast, insect cells or mammalian cells recombination protein expression systems. ScienCell's SUMO protease is fused with a N-terminal His-tag to facilitate its removal from the digested protein sample.

Kit Components

Cat #	Item	Composition	Quantity	Storage
MB9018a	SUMO Protease (5 U/ μ L)	SUMO Protease in: 20 mM Tris-HCl, pH 7.5 150 mM NaCl 1 mM 2-Mercaptoethanol 50% (v/v) glycerol	100 μ l	-80 °C
MB9018c	10X SUMO Protease Buffer with Salt	500 mM Tris-HCl, pH 8.0 2% Igepal (NP-40) 1.5 M NaCl 10 mM DTT	1.25 ml	-20 °C
MB9018d	10X SUMO Protease Buffer without Salt	500 mM Tris-HCl, pH 8.0 2% Igepal (NP-40) 10 mM DTT	1.25 ml	-20 °C

Specifications

Source:	<i>Escherichia coli</i>
Molecular Weight:	27 kDa
Biological Activity:	One unit of SUMO protease is defined as the amount of enzyme needed to cleave 90% of 100 μ g of control SUMO tagged protein at 30 °C for 1 hour.

Quality control

SUMO Protease has greater than 90% single-band purity on SDS-PAGE gel (Figure 1). Representative gel picture shows specific cleavage activity (Figure 2). Non-specific protease activity is undetectable.

Product Use

rScUlp1 is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

Dry Ice. Upon receipt, store SUMO protease (MB9018a) at -80°C and buffers (MB9018c and MB9018d) at -20°C. Aliquot after the first thawing. For convenience, the SUMO protease (MB9018b) may be stored at -20°C for up to 2 months. Please avoid repeated freeze/thaw cycles. Stable for 24 months when stored properly.

Procedure

SUMO protease is active over a wide range of temperatures (2-37 °C), salt concentrations (0-500 mM NaCl), and pH ranges (6-8.5). Performance may be enhanced in the presence of 5-10% glycerol, 0.5-2 mM DTT or 0.01%-0.1% NP40. Researchers will need to optimize their specific reaction conditions. As an initial suggestion, an example of a time course experiment with 1 units of SUMO protease is provided.

1. Prepare cleavage reaction as shown in Table 1.

Table 1. Suggested Reaction System

Component	Volume	Final concentration
SUMO fusion protein	variable	200 µg
SUMO protease	0.8 µL	4 U
10×SUMO Protease Buffer (with salt / without salt)	10 µL	1 x
ddH ₂ O	variable	-
Total volume	100 µL	-

2. Mix and incubate at various temperatures. Remove 5 µl aliquots at each time point as shown in Table 2. Mix the removed aliquots with 15 µl 8M urea and 4 µl 6x Laemmli sample buffer (NOT included) to stop reactions.

Table 2. Recommended sampling times at different temperatures

Temperature	Times
4 °C	2, 16 and 24 hours
16 °C	1, 2, 4 and 8 hours
30 °C	5, 20, 60 and 120 mins

3. Analyze 10 μ l of samples by SDS-PAGE using a suitable gel.

Determine the percent of protein cleavage 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.

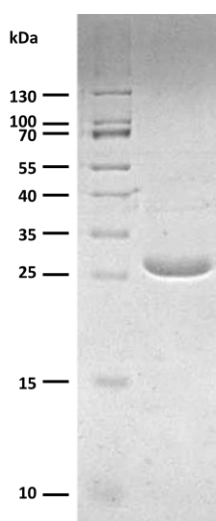


Figure 1. Coomassie staining of SUMO Protease. Recombinant yeast SUMO Protease (4 μ g) resolved on a 16% SDS-PAGE gel under reducing conditions and stained with Coomassie Brilliant Blue G-250.

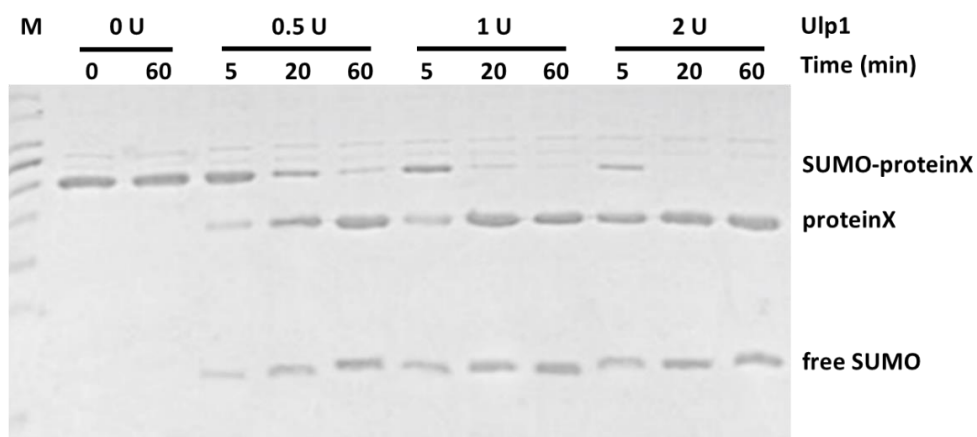


Figure 2. Representative gel picture of SUMO protease activity test. A 100 μ g of control SUMO tagged protein was incubated with various amount of SUMO protease (Ulp1) in a buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.2% NP-40 and 1 mM DTT at 30 $^{\circ}$ C. Samples were taken at indicated time points and quenched by Laemmli sample buffer with 8 M urea. Sample was resolved on a 16% SDS-PAGE gel under reducing conditions and stained with Coomassie Brilliant Blue G-250.