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SUMO Protease

Cat. No. 12588-018 **Size: 250 units**

Description

SUMO Protease, a highly active cysteinyl protease also known as Ulp, is a recombinant fragment of Ulp1 (Ubl-specific protease 1) from *Saccharomyces cerevisiae* (1). SUMO Protease cleaves in a highly specific manner, recognizing the tertiary structure of the ubiquitin-like (UBL) protein, SUMO (2) rather than an amino acid sequence (3). The protease can be used to cleave SUMO from recombinant fusion proteins. The optimal temperature for cleavage is 30°C; however, the enzyme is active over wide ranges of temperature (see table on page 3) and pH (pH 7.0-9.0). Following digestion, SUMO Protease is easily removed from the cleavage reaction by affinity chromatography using the polyhistidine tag at the N-terminus of the protease. SUMO Protease is purified from *E. coli* by affinity chromatography using the polyhistidine tag.

Components

Item	Composition	Amount
SUMO Protease (1 U/µl)	SUMO Protease in: 25 mM Tris-HCl, pH 8.0 1% Igepal (NP-40) 250 mM NaCl 500 µM DTT 50% (v/v) glycerol	5 x 50 µl
10X SUMO Protease Buffer + Salt	500 mM Tris-HCl, pH 8.0 2% Igepal (NP-40) 1.5 M NaCl 10 mM DTT	500 µl
10X SUMO Protease Buffer – Salt	500 mM Tris-HCl, pH 8.0 2% Igepal (NP-40) 10 mM DTT	500 µl

Store SUMO Protease at -20°C (after first-time use) or at -80°C for long-term storage. Avoid multiple freeze/thaw cycles at -80°C. Store 10X SUMO Protease Buffers at 4°C or -20°C.

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For technical questions about this product, call the Invitrogen Tech-Line℠ U.S.A. 800 955 6288

Unit Definition

One unit of SUMO Protease cleaves ≥85% of 2 µg control substrate in 1 h at 30°C.

Unit Assay Conditions

The SUMO Protease assay is performed in 1X SUMO Protease Buffer - Salt (50 mM Tris-HCl, pH 8.0, 0.2% Igepal, 1 mM DTT) with 1 unit enzyme and 2 µg of 85% purified control substrate at 30°C for 1 hour in a total volume of 20 µl.

Guidelines for Cleavage

- For optimal results, perform the cleavage reaction using partially or fully purified recombinant fusion protein.
- For most fusion proteins, SUMO Protease functions optimally in a reaction mixture containing 150 mM NaCl; however, conditions may be optimized by varying the NaCl concentration from 100 mM to 300 mM. Remember to take into account the contribution of salt from the enzyme (*i.e.* 12.5 mM in final buffer) and from your substrate. When setting up your cleavage reaction, use the appropriate 10X SUMO Protease Buffer +/- Salt.
- Keep the imidazole concentration less than 150 mM. Concentrations higher than 150 mM can adversely affect the activity of the protease.

Recommended Conditions for Cleavage of a Fusion Protein

An example of a time course experiment with 10 units of SUMO Protease is provided. If the protein of interest is heat-labile, incubate at 4°C with longer incubation times and/or more enzyme (see table on next page).

1. Add the following to a microcentrifuge tube:

Fusion Protein	20 µg
10X SUMO Protease Buffer +/- Salt	20 µl
Water	to 190 µl
SUMO Protease (10 units)	10 µl
Total volume	200 µl

2. Mix and incubate at 30°C. Remove 20 µl aliquots at 1, 2, 4, and 6 hours.
3. Add 20 µl 2X SDS sample buffer (125 mM Tris-HCl, pH 6.8; 4% SDS; 1.4 M β-mercaptoethanol; 20% (v/v) glycerol; 0.01% bromophenol blue). Keep samples at -20°C until experiment is complete.
4. Analyze 30 µl of sample by SDS-PAGE using a suitable gel.

Determine the percent 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.

Varying Parameters for Cleavage

The percent of 2 µg control substrate hydrolyzed by one unit of SUMO Protease at various temperatures was examined (see table below). More cleaved protein is formed with SUMO Protease by increasing the incubation time. If time is critical, add more SUMO Protease to increase hydrolysis.

	Percentage Substrate Hydrolyzed			
Time	**4°C**	**16°C**	**21°C**	**30°C**
0.5 h	48	73	83	88
1 h	60	87	90	93
2 h	71	94	94	95
3 h	74	95	95	95

Producing Recombinant SUMO Fusion Proteins

To express your gene of interest as a fusion to the SUMO protein, use the Champion™ pET SUMO expression vector (Cat. no. K300-01) available from Invitrogen. Simply use *Taq* polymerase to amplify your gene of interest (beginning at the translation start site), then clone the PCR product into pET SUMO using a 30-minute, room temperature ligation reaction. The pET SUMO vector expresses your protein as an N-terminal SUMO fusion. Once expressed, cleavage with SUMO Protease allows production of native protein, with no extra amino acids added between the cleavage site and the start of your protein.

Removal of SUMO Protease after Cleavage

The SUMO Protease contains a polyhistidine tag at the N-terminus. After cleavage of the fusion protein, remove SUMO Protease from the cleavage reaction by affinity chromatography on a nickel-chelating resin such as ProBond™ Resin (Cat. no. K801-01). Dilute the cleavage reaction in the binding buffer for ProBond™ and perform binding and elution as described in the ProBond™ Purification manual available at www.invitrogen.com. **The cleaved native protein will be in the flow-through fractions.**

Quality Control

SUMO Protease has > 85% single-band purity and must demonstrate functional absence of any non-specific protease activity.

References

1. Li, S.-J. and Hochstrasser, M. (1999) *Nature* 398, 246-251.
2. Müller, S., Hoege, C., Pyrowolakis, G., and Jentsch, S. (2001) *Nature Rev. Mol. Cell Biol.* 2, 202-210.
3. Mossessova, E. and Lima, C.D. (2000) *Mol. Cell* 5, 865-876.

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