Product Information

SUMO Protease, Biotin-tagged

Recombinant protein, aqueous solution, ≥ 25,000 units/mL

SAE0101

Product Description

Synonyms: Small Ubiquitin-like Modifier Protease, ULP, Ubiquitin like protease, Ubiquitin-homology domain protein PIC1, Ubl-specific protease 1.

SUMO proteases are a general class of enzymes that specifically remove the post-translational protein modification (PTM) known as small ubiquitin-related modifier (SUMO), which falls into the PTM class of ubiquitin and/or ubiquitin-like proteins (UBL).[1] The enzyme commonly referred to as 'SUMO protease' is the Ubl-specific protease 1 (Ulp1) from *Saccharomyces cerevisiae*. This was the first of this class of enzymes to be isolated.[2]

SUMO protease specifically cleaves the SUMO moiety in a 'scarless' manner. SUMO protease recognizes the tertiary structure of the Ubiquitin-like SUMO domain and hydrolyzes the peptide bond in the x–Gly–Gly–x sequence after the Gly-Gly bond, at the C-terminus of the SUMO domain.[3] In addition to cleavage of natural SUMO-modified proteins, SUMO protease is used to cleave recombinant SUMO fusion proteins. The SUMO domain is a known solubility-enhancing fusion tag that is used in recombinant protein expression.[4]

This biotin-tagged SUMO protease is designed to be used for on-column cleavage of SUMO fusion proteins. This method specifically cleaves the protein of interest from a column-bound SUMO fusion protein, leaving the SUMO domain bound to the affinity column (such as a Ni-NTA column) and eluting only the protein of interest. On-column cleavage is advantageous over post-elution cleavage for several reasons:

- This eliminates most of the impurities normally associated with purification on Ni-chelating columns.
- This also allows much gentler elution conditions, with an added flexibility in the composition of the elution buffer. This can help to prevent protein aggregation and inactivation.

Following cleavage, the protease can be efficiently removed by using any avidin-conjugated or streptavidin-conjugated beads.

This biotin-tagged SUMO protease has been enzymatically biotinylated without affecting its proteolytic activity. It does not include any additional protein purification tags, such as a histidine-tag or a GST tag.

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Product

This product is supplied at a concentration of ≥ 25,000 U/mL in an aqueous buffer that contains 20 mM Trizma®, pH 8.0, 2 mM DTT, 50 mM NaCl, and 50% (v/v) glycerol.

Unit definition: One unit is defined as the amount that will cleave 90% of 100 pmol of SUMO-GST in 1 hour at 30 °C.

Storage/Stability

Store this liquid product at –20 °C. The product is expected to retain activity for at least 2 years when stored at –20 °C.

Procedure

SUMO protease is active over a wide range of temperatures (2-30 °C), ionic strengths (0-400 mM NaCl), and pH ranges (6-8.5). However, its activity may vary depending on the substrate and conditions. Researchers will need to optimize their specific reaction conditions.

As an initial suggestion, 20 units of SUMO protease can be used per mg of target protein for 1 hour at 30 °C, or overnight at 2-8 °C. The cleavage efficiency can then be estimated by SDS-PAGE. If necessary, the amount of SUMO protease can then be adjusted.

SUMO protease works better in the presence of reducing agents, such as 0.5-2 mM DTT. DTT in the reaction mixture can significantly enhance cleavage efficiency, especially during longer incubations.

To perform on-column cleavage:

1. Dilute the desired amount of SUMO protease in a volume equal to one column volume.
2. Inject the protease solution directly into the column.
3. Incubate the column at the desired temperature and time according to the previous guidelines.
4. Elute the cleaved target protein with 1-3 column volumes, depending on the required protein concentration.
5. If the target protein is prone to precipitation at higher concentrations, elution can be performed by continually circulating the protease solution in a larger volume through the column in a closed circle, until all target protein is removed from the column.

References

1. Hickey, C. M. *et al.*, *Nat. Rev. Mol. Cell Biol.*, 13(12), 755-766 (2012).
2. Li, S. J., and Hochstrasser, M., *Nature*, 398(6724), 246-251 (1999).
3. Li, S. J., and Hochstrasser, M., *J. Cell Biol.*, 160(7), 1069-1082 (2003).
4. Kimple, M. E. *et al.*, *Curr. Protoc. Protein Sci.*, 24, 73:Unit 9.9 (2013).

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