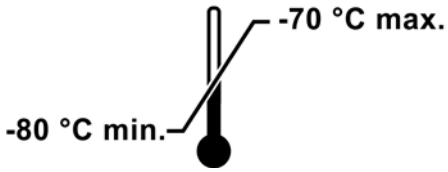
SUMO Express Protease

Catalog No. 30801-2

Store at –80 °C.



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Product Guarantee: Lucigen guarantees that this product will perform as specified for one year from the date of shipment. Please avoid using reagents for greater than one year from receipt.

Technical Specifications

Product Description	SUMO Express Protease: 1 Unit/µl; 200 U (4 x 50 U).
Storage Buffer	50% glycerol, 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.5 mM DTT, 1% Triton X-100.
Stability	SUMO Express Protease is stable for one year from the date received if stored at –80 ºC.
Recommended Reaction Conditions	SUMO Express Protease at 1 unit per 10-100 µg SUMO fusion protein, 150 mM NaCl, 20 mM Tris-HCl pH 8.0, 2 mM DTT.
Activity Determination	One unit will cleave ≥90% of 100 µg of the SUMO Cleavage Control Protein in 30 minutes at 4 °C in 10% glycerol, 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 2 mM DTT.
Purity	≥95% pure by SDS-PAGE and RP-HPLC.

Description

SUMO Express Protease is a highly active and robust engineered protease derived from the yeast *ULP1* gene. It is used to remove the SUMO tag from recombinant SUMO-tagged fusion proteins after purification. It has been specifically engineered to recognize and cleave the stabilized form of the SUMO tag used in Lucigen's Expresso™ SUMO Cloning and Expression kits. This stabilized SUMO tag contains amino acid substitutions that render it resistant to desumoylation enzymes present in eukaryotic cells, enabling expression of SUMO fusion proteins in insect or mammalian

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cells. The SUMO Express Protease contains compensatory mutations that enable it to recognize and cleave the stabilized SUMO tag. Other commercially available SUMO proteases lacking these compensatory mutations cannot cleave the SUMO tag used in the Expresso SUMO kits. SUMO Express Protease exhibits activity against the native (nonstabilized) SUMO tag, but at a reduced rate compared to native SUMO protease. SUMO Express protease is 6xHis tagged, allowing convenient removal of the protease after cleavage by metal affinity chromatography.

SUMO Express Protease is active under a variety of buffer conditions, allowing flexibility in cleavage protocols. The protease is tolerant to many common buffer additives, including salt, non-ionic detergents, imidazole, and low concentrations of urea or guanidine (1). The optimal temperature for cleavage is 30 °C, but the protease is active from 4 °C to 37 °C. The pH optimum for cleavage is 8.0, but the range 6.0 to 10.0 is tolerated. Since each SUMO fusion protein behaves uniquely, we recommend performing test cleavage reactions on a small scale. For most fusion proteins, 1 unit of SUMO Express Protease will be sufficient to digest 10 -100 µg in 1 hour at 30 °C under the recommended conditions.

Protocol: Removal of SUMO tags from fusion proteins using SUMO Express Protease

SUMO Express Protease is used to remove the SUMO tag from recombinant SUMO-tagged fusion proteins after purification. SUMO fusion proteins expressed using Lucigen's Expresso SUMO Cloning and Expression kits have an amino terminal 6xHis peptide to enable rapid purification by immobilized metal affinity chromatography (IMAC). After cleavage of the purified fusion protein, the released protein of interest is easily separated from the SUMO tag and SUMO Express Protease by use of the 6xHis tag present on both the protease and the cleaved SUMO tag. The cleavage mixture is simply applied to an IMAC column, and the free target protein recovered in the flow-through; the SUMO tag and SUMO Express Protease remain bound to the IMAC matrix. Although SUMO Express Protease is tolerant to imidazole up to 300 mM, residual imidazole from initial purification of the SUMO fusion protein will interfere with binding of the protease and the cleaved SUMO tag to the IMAC resin. Dialysis is recommended before cleavage to remove imidazole and to exchange the fusion protein into the desired buffer for cleavage.

Dialysis. Dialyze the purified SUMO fusion protein for 24 hours at 4 °C to remove imidazole. The recommended buffer for dialysis is (20 mM Tris-HCl, 150 mM NaCl, 10% glycerol, pH 8.0). Alternative buffers (e.g. phosphate, HEPES) are also tolerated by the protease. For maximal activity maintain pH in the range 7 to 9. Salt concentrations of 500 mM and higher are detrimental. Non-ionic detergents such as Triton X-100 or Igepal (NP-40) may be used at 1%.

Cleavage. The recommended buffer for cleavage is the same as the dialysis buffer, with the addition of 2 mM fresh DTT. We suggest performing small-scale test reactions to evaluate cleavage efficiency with each different fusion protein. Add 1 unit of protease per 10-100 µg of fusion protein. Incubate at 30 °C for 1 hour, or at 4 °C overnight. To evaluate cleavage, remove a sample of the reaction (5-10 µg of fusion protein). Add an equal volume of 2X SDS-PAGE loading buffer, heat to 95 °C for 5 minutes and run on SDS-PAGE along with molecular weight markers. The free 6xHis-SUMO tag has an expected molecular weight of 12 kDa, but migrates at ~15-18 kDa. If partial cleavage is observed, another aliquot of SUMO Express Protease may be added and digestion continued at 4 °C overnight.

Recovery of cleaved target protein. After the cleavage reaction is complete, the SUMO tag and SUMO Express Protease, as well as any residual uncleaved fusion protein, are removed from the sample by adsorption to metal affinity chromatography (IMAC) matrix. The sample can be applied directly to an IMAC column. The released protein will be present in the column flowthrough and wash, while the 6xHis tagged SUMO fragment and protease remain bound to the column. **Note:** In some cases the presence of 2 mM DTT in the cleavage reaction may cause reduction of metal

ions in the IMAC resin, resulting in leaching of the metal and failure to retain 6xHis-tagged proteins. We recommend testing the IMAC resin with the cleavage buffer before applying the digested sample to the resin. IMAC resins that have been reduced turn brown in color. If necessary, dilute the cleavage reaction to reduce the DTT concentration.

See the Expresso SUMO Cloning and Expression System Manuals for additional information on expression of SUMO fusion proteins and cleavage with SUMO Express Protease.

References

1. Malakhov, M.P., Mattern, M.R., Malakhova, O.A., Drinker, M., Weeks, S.D. and Butt., T.R. (2004). SUMO fusions and SUMO-specific protease for efficient expression and purification of proteins. *J Struct Funct Genomics.* **5**, 75.

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