SUMOstar Protease 1 (Cat. No. 4110)

Description and Application

SUMOstar Protease, a highly active and robust recombinant protease, cleaves SUMOstar from recombinant fusion proteins. Unlike thrombin, EK, or TEV protease, whose recognition sequences are short and degenerate, SUMOstar Protease recognizes the tertiary structure of SUMOstar, with subsequent cleavage at the Gly-Gly sequence of the recombinant fusion protein of interest (POI). As a result, SUMOstar Protease does not cleave within the POI, while leaving the desired N-terminus. SUMOstar Protease 1 contains a polyhistidine tag at the N-terminus, allowing easy removal from the cleavage reaction by affinity chromatography. The protease cleaves consistently over a broad range of temperature (30°C is optimal), pH [5.5 – 9.5], and ionic strength.

Components

Units: 250, 500, 1000, 5000 units

Unit Definition

One unit of SUMOstar Protease 1 cleaves 90% of 100 µg of SUMOstar Control Protein 1 (1 h at 30°C).

Buffer: 25 mM Tris-HCl, pH 7.5
150 mM NaCl
2mM DTT
10% glycerol

Storage: For short-term use, store at +4° C. Long-term storage should be at -80° C. Avoid multiple freeze/thaw cycles.

Protocol

1. Dialyze the IMAC purified SUMOstar fusion proteins (4°C) against an appropriate physiological buffer (e.g. 20 mM Tris-HCl, 150 mM NaCl, pH 8.0). If the dialysis volume does not exceed >100-fold sample size, multiple buffer changes (each 4h or greater) should be employed to effectively remove salts or detergents.

2. Add SUMOstar Protease (1U per 100 µg of substrate) and incubate at 30°C for 1 h in the presence of 1-5mM dithiothreitol (DTT).

3. In addition, the following guidelines may be helpful:

   i. If your protein of interest is sensitive to reducing agents, a less aggressive agent (e.g. BME or TCEP) can be used. Longer incubation times may be required.
   ii. If low cleavage efficiency is observed, consider increasing the time and/or amount of SUMOstar protease. Overnight at 4°C, for example, may be convenient.
   iii. Consider adding SUMOstar protease incrementally throughout the time course of the reaction.
   iv. If a fraction of the fusion protein is mis-folded or aggregated, it may be resistant to digestion.

References

Marblestone JG, Edavettal SC, Lim Y, Lim P, Zuo X, Butt TR (2006). " Comparison of SUMO fusion technology with traditional gene fusion systems: Enhanced expression and solubility with SUMO," Protein Science, 15:182-9.

Butt TR, Edavettal SC, Hall JP, Mattern MR (2005). " SUMO fusion technology for difficult-to-express proteins," Protein Expr Purif., 43(1):1-9.

Zuo X, Li S, Hall J, Mattern MR, Tran H, Shoo J, Tan R, Weiss SR, Butt TR (2005). "Enhanced expression and purification of membrane proteins by SUMO fusion in Escherichia coli.," J Struct Funct Gen., 6:103-11.

Zuo X, Mattern MR, Tan R, Li S, Hall J, Sterner DE, Shoo J, Tran H, Lim P, Sarafianos SG, Kazi L, Navas-Martin S, Weiss SR, Butt TR (2005). "Expression and purification of SARS coronavirus proteins using SUMO-fusions," Protein Expr Purif., 42(1):100-10.

Malakhov MP, Mattern MR, Malakhov OA, Drinker M, Weeks SD, Butt TR (2004). "SUMO fusions and SUMO-specific protease for efficient expression and purification of proteins," J Struct Funct Gen, 5:75-86.

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