SUMO Protease

Introduction:

SUMO protease, also known as Ulp, is a highly active cysteine protease derived from a recombinant gene fragment of Ubl-specific protease 1 in Saccharomyces cerevisiae. SUMO proteases recognize and cleave peptide bonds after the SUMO carboxyl (C-terminal) of the Ubiquitin-like (UBL) protein in a highly specific manner. SUMO is an Ubiquitin-like protein that is commonly found in post-translation modification (PTM), which plays an important role in the stability of proteins and the regulation of biological functions.

The optimal lysis temperature for SUMO proteases is 30 ºC and the optimal pH is 8.0, but it is in the wide pH range (6.0-10.0) and temperature range (2 to 30 ºC). The ion strength range (0-400mM NaCl) maintains high enzyme activity. In special scenario use, the sample can be digested overnight at 4ºC to maintain the structure and biological activity of the protein of interest. SUMO Protease has higher digestion activity in the presence of the reducing agent DTT (0.5~2mM), and the addition of DTT at the appropriate concentration to the digestion system can significantly improve the digestion efficiency, especially during long-term digestion, such as Enzymes are cut overnight at 4 ºC. The lysed SUMO protease can be removed using histidine tags at its N-terminus for affinity chromatography.

Raw materials/composition

Component	Detailed composition	Specification		
		200U	1000U	5000U
SUMO Protease (10U/μl)	25 mM Tris-HCl, pH 8.0 0.1% Igepal (NP-40) 250 mM NaCl 500 μM DTT 50% (v/v) glycerol	20 μl	100 μl	500 μl
10X SUMO Protease Buffer + Salt	500 mM Tris acetate, pH 8.0 2% Igepal (NP-40) 1.5 M NaCl 10 mM DTT	400 μl	2×1 ml	10×1 ml
10X SUMO Protease Buffer – Salt	500 mM Tris acetate, pH 8.0 2% Igepal (NP-40) 10 mM DTT	400 μl	2×1 ml	10×1 ml

Store conditions

Long-term storage: -80 °C

Experimental operation

Since different proteins have different properties, it is recommended to optimize the ratio of enzymes to proteins when used, and the following is a simple protocol for enzymatic reactions for most types of recombinant fusion proteins.

1. The centrifuge tube is configured with the following reaction system in 1.5ml (For example, in a 200 μl system, either unsalted and salted buffers can be used in parallel with one or both).

Component	Size
SUMO-tag Protein	20μg
SUMO Protease (10U/μl)	1μl
10X Reaction Buffer –/+ Salt	20μl
H2O	To 200μl
Total	200μl

2. Mix incubation at 30ºC for 1h, 2h, 4h and 6h. If the protein is unstable at high temperatures, the 4ºC digestion reaction can be done overnight (about 16 h). You can also optimize the reaction conditions according to the table below:

Temperature	Reaction Time
4°C	15-16h
16°C	4h
25°C	1.5h
30°C	1h

3. Take 20 μl of the digestion product from the different time points above for SDS-PAGE electrophoresis analysis to determine the optimal enzymatic reaction conditions required for the reaction.
4. Perform the amplified digestion reaction according to the optimal enzyme concentration and reaction time obtained by the experiment.
5. His-tagged SUMO and SUMO proteases are removed using affinity chromatography.

Note

1. For best digestion, the recombinant protein must be a purified protein.
2. For most fusion proteins, the concentration of NaCl in the SUMO Protease reaction system is 150mM. However, the concentration of NaCl can be adjusted between 0 and 300 mM according to the actual situation to achieve the best digestion effect.
3. The final concentration of imidazole in the digestion reaction should not be higher than 150 mM, otherwise the digestion efficiency of SUMO Protease may be affected.
4. This product is for scientific use only.