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 Protease recognizes and cleaves 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 30ºC and the optimal pH is 8.0, but it can also maintain high enzyme activity at pH range (6.0-10.0), temperature range (2-30ºC) and ion strength range (0-400 mM NaCl). Under some circumstances, the sample can be digested overnight at 4ºC to maintain the structure and biological activity of the target protein. SUMO Protease has higher digestion activity in the presence of the reducing agent DTT (0.5-2 mM), and adding appropriate concentration of DTT to the enzyme digestion system can significantly improve the digestion efficiency, especially during extended digestion, such as enzymes digestion overnight at 4ºC. The lysed SUMO protease can be removed using histidine tags at its N-terminus for affinity chromatography.

Components

Components	Ingredients	Specification		
		200 U	1000 U	5000 U
SUMO Protease (10 U/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 x 1 mL	10 x 1 mL
10X SUMO Protease Buffer – Salt	500 mM Tris acetate, pH 8.0 2% Igepal (NP-40) 10 mM DTT	400 μL	2 x 1 mL	10 x 1 mL

Storage Condition

Long-term storage: -80°C

Experiment Procedures

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

1. 200 μL reaction system (unsalted and salted buffers can be both used in parallel, or just choose one buffer to use):

Components	Size
SUMO-tag Protein	20 μg
SUMO Protease (10 U/μL)	1 μL
10X Reaction Buffer –/+ Salt	20 μL
H_2O	To 200 μL
Total	200 μL

2. Incubate 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 16h). You can also optimize the reaction conditions as follows:

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

3. Take 20 μL of the digests from the different time points above, and carry out 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 results, the recombinant protein must be a purified protein.
2. For most of the fusion proteins, the concentration of NaCl in the SUMO Protease reaction system is 150 mM. However, the concentration of NaCl can be adjusted between 0 and 300 mM to achieve the best digestion effect.
3. The final concentration of imidazole in 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.