SUMO Protease

REF: EG26312S

Storage Condition

Store at -85℃ to -65℃ for 2 years. Aliquot storage is recommended to avoid repeated freezing and thawing.

Components

Component	Amount
SUMO Protease (1 U/μl)	200 μl
10× SUMO Buffer	2×1.5 ml

Description

SUMO Protease is a recombinant protein obtained by expressing the ULP1 (Ubl-specific protease 1) from Saccharomyces cerevisiae in E. coli. SUMO protease specifically recognizes the three-dimensional structure rather than the linear amino acid sequence of SUMO (Small Ubiquitin-like Modifier), and cleaves the peptide bond after Gly-Gly within the x-Gly-Gly-x motif at the C-terminus of SUMO. Based on this property, SUMO protease offers two major advantages over proteases such as TEV protease and thrombin:After cleavage, the target protein retains its native N-terminus without any extra amino acids, which is the greatest advantage over TEV protease and thrombin.It recognizes a long sequence, giving it very high specificity.

SUMO protease is active over a wide ranges temperature (4~30℃), ionic strength (0~300 mM NaCl), imidazole concentration (0~150 mM), reducing-agent (0~8 mM DTT/TCEP), and pH (pH6.0~9.5). In addition, this product carries a polyhistidine (His) tag, which facilitates removal of SUMO protease by affinity chromatography to purify the target protein.

Definition of Activity Unit

One unit (U) is defined as the amount of enzyme that cleaves >85% of 2 μg of substrate in 1× SUMO Buffer at 30℃ for 1 h.

Storage Buffer

25 mM Tris-HCl; 250 mM NaCl; 0.5 mM DTT; 0.1% NP-40; 50% Glycerol; pH 8.0 @ 25℃。

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Non-specific Protease Activity

A 50 μl reaction, 1 U of SUMO Protease was incubated with 3 μg of a standard protein for 4 hours at 37℃ , and analyzed by SDS-PAGE; the protein mixture showed no degradation.

Protocol

1. Combine the following components on ice in the following order:

Reagent	Amount
Fusion protein	≤600 pmol[a]
10× SUMO Buffer	10 μl
SUMO Protease (1 U/μl)	1 μl
ddH_2O	Up to 100 μl

a. For a 50 kDa protein, 600 pmol of fusion protein corresponds to 30 μg.

2. Incubate at 30℃ for 1 h or at 4℃ overnight (about 16 h). Keeping the reaction temperature constant, appropriately extending the incubation time can improve cleavage efficiency.

3. Add 33 μl of Protein Loading Buffer (SDS, 4×) (REF: EG21323S) to the EP tube, boil the sample for 5 min, and take 10 μl for SDS-PAGE analysis.

4. Based on the cleavage result, optimize the amount of SUMO protease, incubation temperature, and incubation time to determine the optimal reaction conditions.

Notice

1. For best cleavage, ensure that the recombinant protein is partially or fully purified.

2. The imidazole concentration in the reaction should less than 150 mM; otherwise SUMO protease activity will be inhibited.

3. SUMO protease shows optimal activity at 100 mM NaCl, but this can be adjusted within 0~300 mM as needed to achieve the best cleavage.

4. SUMO protease remains active in reaction systems containing 0~8 mM DTT/TCEP (final concentration).

5. The optimal reaction temperature of SUMO protease is 30℃ , but it is active within 4~30℃ ; lower temperatures require longer incubation. The reaction temperature can be flexibly adjusted according to the requirements of the target protein.

6. If conditions permit, reducing the reaction volume can improve cleavage efficiency; however, attention should be paid to the NaCl and imidazole ranges mentioned in notes 2 and 3.

7. For your safety and health, please wear a lab coat and disposable gloves during operation.

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