SUMO Protease

User Manual

384 Oyster Point Blvd, Suite 15
South San Francisco, CA 94080
Phone: 1 (888) MCLAB-88
Fax: 1 (650) 872-0253
www.mclab.com

Cat # (SP-100, SP-200, SP-300, SP-400)

Description

SUMO Protease, a highly active cysteinyl protease also known as Ulp, is a recombinant fragment of Ulp1 (Ubl-specific protease 1) from Saccharomyces cerevisiae. SUMO Protease cleaves in a highly specific manner, recognizing the tertiary structure of the ubiquitin-like (UBL) protein, SUMO rather than an amino acid sequence. The protease can be used to cleave SUMO from recombinant fusion proteins. The optimal temperature for cleavage is 30°C; however, the enzyme is active over wide ranges of temperature and pH (pH 7.0–9.0). Following digestion, SUMO Protease is easily removed from the cleavage reaction by affinity chromatography using the polyhistidine tag at the N-terminus of the protease. SUMO Protease is purified from E. coli by affinity chromatography using the polyhistidine tag.

Guidelines for Cleavage

- For optimal results, perform the cleavage reaction using partially or fully purified recombinant fusion protein.
- For most fusion proteins, SUMO Protease functions optimally in a reaction mixture containing 150 mM NaCl; however, conditions may be optimized by varying the NaCl concentration from 100 mM to 300 mM. Remember to take into account the contribution of salt from the enzyme (i.e. 12.5 mM in final buffer) and from your substrate. When setting up your cleavage reaction, use the appropriate 10X SUMO Protease Buffer +/- Salt.
- Keep the imidazole concentration less than 150 mM. Concentrations higher than 150 mM can adversely affect the activity of the protease.

Recommended Conditions for Cleavage of a Fusion Protein

An example of a time course experiment with 10 units of SUMO Protease is provided. If the protein of interest is heat-labile, incubate at 4°C with longer incubation times and/or more enzyme.

1. Add the following to a microcentrifuge tube:
Fusion Protein 20 µg
10X SUMO Protease Buffer +/- Salt 20 µl
Water to 190 µl
SUMO Protease (10 units) 10 µl
Total volume 200 µl

2. Mix and incubate at 30°C. Remove 20 µl aliquots at 1, 2, 4, and 6 hours.

3. Add 20 µl 2X SDS sample buffer (125 mM Tris-HCl, pH 6.8; 4% SDS; 1.4 M β-mercaptoethanol; 20% (v/v) glycerol; 0.01% bromophenol blue). Keep samples at –20°C until experiment is complete.

4. Analyze 30 µl of sample by SDS-PAGE using a suitable gel.

Determine the percent protein cleavage by analyzing the amount of cleaved products formed and amount of uncleaved protein remaining after di gestion. After evaluating the initial results, you may optimize the cleavage reaction for your specific protein by optimizing the amount of SUMO Protease, incubation temperature, or reaction time.

Varying Parameters for Cleavage

The percent of 2 µg control substrate hydrolyzed by one unit of SUMO Protease at various temperatures was examined (see table below). More cleaved protein is formed with SUMO Protease by increasing the incubation time. If time is critical, add more SUMO Protease to increase hydrolysis.

Percentage Substrate Hydrolyzed

Time	4°C	16°C	21°C	30°C
0.5 h	48	73	83	88
1 h	60	87	90	93
2 h	71	94	94	95
3 h	74	95	95	95

References

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