

Manual

Product name: SUMO Protease

Cat #: SP-100, SP-200, SP-OEM, B-SP10S, B-SP10F

Description:

SUMO Protease (also known as Ulp) is a cysteinyl protease with very high activity. It is a recombinant fragment of Ulp1 (Ubl-specific protease 1) originated from *Saccharomyces cerevisiae* and purified from *E. coli* by affinity chromatography using the polyhistidine tag. SUMO Protease recognizes the tertiary structure of the ubiquitin-like (UBL) protein small ubiquitin modifier (SUMO) rather than the amino acid sequence, so it cleaves with a high structural specificity. SUMO protease can be used to cleave SUMO tags from recombinant fusion proteins. The enzyme is active over extensive temperature ranges from 4°C to 30°C and pH ranges from 7.0 to 9.0. The optimal temperature for cleavage is 30°C and SUMO Protease is easy for removal from the cleavage reaction by affinity chromatography using its polyhistidine tag at the N-terminus.

Protocol:

Recommended Conditions for Cleavage of a Fusion Protein.

An example of a time course experiment with 100 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 200 µg
10X SUMO Protease Buffer +/- Salt 20 µl
SUMO Protease (100 units/µl) 1 µl
Add water to a total volume of 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 of protein cleavage by analyzing the amount of cleaved products formed and amount of uncleaved protein remaining after digestion. 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.