Product Information

SUMO Protease Kit

Kit Contents

Component	29139-250U	29139-1000U	29139-5000U
29139A: SUMO Protease (5 U/uL)	250 units	1,000 units	5,000 units
29139B: 10X Reaction Buffer -Salt	1 mL	1 mL	1 mL
29139C: 10X Reaction Buffer +Salt	1 mL	1 mL	1 mL
N006-INT: Control Substrate (1 mg/mL)	10 ug	50 ug	100 ug

Storage and Handling

Store SUMO Protease at -80°C for long-term storage. Avoid multiple freeze/thaw cycles at -80°C. After the first use, store SUMO Protease at -20°C. Aliquoting of SUMO Protease is not required after first use; the solution contains 50% glycerol and will not freeze at -20°C. Store 10X Reaction Buffers and Control Substrate at -20°C. Product is stable for at least 24 months from date of receipt when stored as recommended.

SUMO Protease is supplied as a 0.2 um-filtered solution containing 5 U/uL SUMO Protease, 20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 0.5 mM DTT, 50% glycerol, pH 8.0.

10X Reaction Buffer -Salt is supplied as a 0.2 um-filtered solution containing 500 mM Tris-HCl, 2% IGEPAL® (NP-40), 10 mM DTT, pH 8.0.

10X Reaction Buffer +Salt is supplied as a 0.2 um-filtered solution containing 500 mM Tris-HCl, 2% IGEPAL® (NP-40), 10 mM DTT, 1.5 M NaCl, pH 8.0.

Control Substrate is supplied at 1 mg/mL as a 0.2 um-filtered solution containing 20 mM HEPES, 500 mM NaCl, 1 mM TCEP, pH 7.0.

Note: 10 ug of Control Substrate is enough to perform a 50 uL reaction as an optional positive control for SUMO Protease activity. Control Substrate does not need to be used every time SUMO Protease is used as long as the expected cleavage of your substrate of interest is observed.

Molecular Weight:

Recombinant His-tagged SUMO Protease: 30 kDa, observed by reducing SDS-PAGE.

Control Substrate: 29 kDa (before cleavage), runs at ~35 kDa on reducing SDS-PAGE; 13.7 kDa and 15.3 kDa (after SUMO Protease cleavage), runs at ~16 kDa and ~18 kDa on reducing SDS-PAGE.

Purity (SUMO Protease): >95% pure as demonstrated by reducing SDS-PAGE.

Activity (SUMO Protease): 5 U/uL

Unit Definition: One unit of SUMO Protease cleaves >85% of 2 ug of Control Substrate in 1 hour at 30°C in 1X Reaction Buffer with or without salt.

Product Description

SUMO Protease is a highly sequence-specific cysteine protease that recognizes the tertiary structure of SUMO protein. SUMO Protease efficiently and specifically cleaves the SUMO protein from recombinant fusion proteins. The optimal temperature for cleavage is 30°C, but the reaction can be performed at temperatures as low as 4°C.

Biotium's recombinant SUMO Protease is expressed from *E. coli* with a His-tag for easy removal from the reaction using nickel affinity chromatography.

The SUMO Protease Kit includes Control Substrate as an optional reaction control. It is a His-tagged SUMO fusion protein with a molecular weight of 35 kDa before cleavage, observed by 4-20% reducing SDS-PAGE (Figure 1). When Control Substrate is cleaved with SUMO Protease, two bands at ~16 kDa and ~18 kDa are observed under 4-20% reducing SDS-PAGE.

Experimental Protocol

Optimal incubation times and enzyme concentrations may vary between substrates and should be determined empirically. As a starting point, we recommend using 5 units of SUMO protease per 10 ug substrate. Reactions may be scaled up linearly. Typical reaction conditions for cleaving Control Substrate in a 50 uL reaction are provided below.

Note: Control Substrate is provided as an optional positive control for SUMO Protease activity. Control Substrate does not need to be used every time SUMO Protease is used as long as the expected cleavage of your substrate of interest is observed.

Table 1: Control Reaction Setup

Component	Stock concentration	Volume	Final concentration (50 uL reaction)
Water	N/A	34 uL	N/A
Reaction Buffer	10X	5 uL	1X
Control Substrate	1 mg/mL	10 uL	0.2 mg/mL (10 ug total)
SUMO Protease	5 U/uL	1 uL	0.1 U/uL (5 U total)

1. Combine the reaction components in the order shown in Table 1.
2. Incubate at 30°C for 1 hour or at 4°C overnight.
3. Optional: Isolate the cleaved substrate by running the sample reaction over a nickel affinity column and collect the flow through. The SUMO Protease and SUMO tag will remain bound to the column. The tagless Control Substrate will be in the flow-through. Alternatively, the reaction can be analyzed by SDS-PAGE without column clean-up.
4. Reaction efficiency of SUMO Protease can be assessed by running a reducing SDS-PAGE of Control Substrate in the presence and absence of SUMO Protease. The cleaved Control Substrate will produce bands at ~16 kDa and ~18 kDa. Uncleaved Control Substrate will run at 35 kDa. If SUMO Protease is not removed by nickel column, it will be present on the gel at 30 kDa (Figure 1).

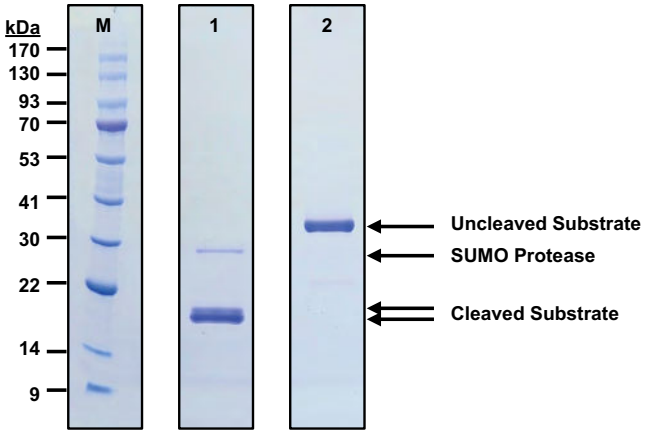

Figure 1. Reducing SDS-PAGE and One-Step Blue® staining of Control Substrate (2 ug) in the presence (Lane 1) or absence (Lane 2) of SUMO Protease. Samples were incubated at 30°C for 1 hour prior to gel analysis.

Related Products

Cat. No.	Product
21003-1L	One-Step Blue® Protein Gel Stain
21004	One-Step Lumitein™ Protein Gel Stain
21005	One-Step Lumitein™ UV Protein Gel Stain
40136	4X Protein Loading Buffer with Orange Tracking Dye
21530	Peacock™ Prestained Protein Marker
21531	Peacock™ Plus Prestained Protein Marker
29090	TEV Protease Kit
41024-1L	Water, Ultrapure Molecular Biology Grade

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