GeneCopoeia™
Expressway to Discovery

Protocol • CoolCutter™ SUMO Protease • Catalog Number SUMO-1000

Efficient cleavage of SUMO-tagged fusion proteins

Description

The **S**mall **U**biquitin **MO**difier (SUMO) gene fusion system allows for the efficient removal of SUMO tags. The CoolCutter™ SUMO Protease product is a mixture of recombinant human and mouse SUMO proteases which delivers superior SUMO cleavage activity with both the native sumoylated proteins and the SUMO tag in recombinant SUMO fusion proteins. The CoolCutter enzyme recognizes the tertiary structure of SUMO rather than an amino acid sequence for a clean release of the desired peptides. Its high activity in a wide range of pH, salt and detergent concentrations makes it highly suitable for recombinant protein expression and purification.

CoolCutter SUMO protease can cleave any protein that is fused to the C-terminus of SUMO except for fused proteins beginning with proline. Cleavage efficiency of CoolCutter SUMO Protease is low for those SUMO-fused proteins that begin with luecine, lysine or valine.

CoolCutter SUMO Protease is ideal for use with OmicsLink™ expression-ready clones, particularly the bacterial pReceiver B12 and pReceiver B13 vectors.

Contents and storage

1000 Units

CoolCutter SUMO protease is provided with 10X high-salt and 10X no-salt buffers.

Store at –80°C. Avoid repeated freeze-thaw cycles. CoolCutter SUMO protease is stable for 18 months or longer at –80°C.

Quality control

Non-specific protease activity is undetectable.

Unit Definition

One unit of CoolCutter SUMO protease is defined as the amount of enzyme needed to cleave 90% of 5 µg of SUMO-eGFP substrate protein at 30°C in 60 minutes.

Applications

1) Removal of SUMO from native sumoylated proteins and SUMO tag from SUMO fusion (or SUMO-containing) recombinant proteins.
2) Purification of XXX-SUMO tagged recombinant protein with XXX is a tag such as MBP, AviTag™ 6xHIS that allows affinity binding.

Related products

OmicsLink Expression-Ready Bacterial Clones, B12 and B13
Positive control substrates for CoolCutter SUMO protease
SUMO monoclonal antibody

Continued on backside

Suggested protocol

1. Following purification of SUMO-tagged fusion protein, resuspend the protein at 4°C in either PBS (pH 7.4) or 20 mM TRIS buffer containing 150 mM NaCl, pH 8.0.

2. Add CoolCutter SUMO protease to the substrate. Use 1 unit enzyme for every 2 µg substrate. Adjustment of the amount of protease may be required depending on different SUMO fusion proteins. Add DTT to a final concentration of 2 mM.

3. Incubate the reaction at 35°C for 30 minutes with gentle agitation or incubate overnight at 4°C to 15°C.. Do not vortex.

4. Check completion of reaction using SDS-PAGE. Adjust enzyme amount to achieve desired cleavage.

Buffer compositions

10X SUMO protease buffer (high-salt)
500 mM Tris-HCl, pH8.0
2% Igepal (NP-40)
1.5 M NaCl
10 mM DTT

10× SUMO protease buffer (no salt)
500 mM Tris-HCl, pH8.0
2% Igepal (NP-40)
10 mM DTT

SUMO protease storage buffer
25 mM Tris-HCl, pH8.0
1% Igepal (NP-40)
250 mM NaCl
50 uM DTT
50% (v/v) glycerol

References

Li, SJ et al. (1999) A new protease required for cell-cycle progression in yeast, Nature, 398(6724):246-51.

Schwarz, SE et al. (1998) The ubiquitin-like proteins SMT3 and SUMO-1 are conjugated by the UBC9 E2 enzyme, Proc. Natl Acad Sci USA, 95(2):560-4.

Mossessova, E et al. (2000) Ulp1-SUMO crystal structure and genetic analysis reveal conserved interactions and a regulatory element essential for cell growth in yeast, Mol Cell, 5(5):865-76.

GeneCopoeia, Inc.
9620 Medical Center Drive, #101
Rockville, Maryland 20850
Tel: 301-762-0888 Fax: 301-762-8333
Email: inquiry@genecopoeia.com
Web: www.genecopoeia.com