User manual

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

# CoolCutter™ SUMO Protease

## ■ Components

**Cat.No: PE001 Size: 200 Units**

| Cat.No | Component | Concentration | Volume |
|---|---|---|---|
| PE001-01 | CoolCutter™ SUMO Protease | 2U/μL | 100 μL |
| PE001-02 | 10× SUMO Protease Buffer (with salt) | 10× | 1 mL |
| PE001-03 | 10× SUMO Protease Buffer (without salt) | 10× | 1 mL |
| PE001-04 | Positive Control | 2 mg/mL | 50 μL |

Store CoolCutter™ SUMO protease at –20°C (after initial use) or at -80°C for long-term storage. Avoid repeated freeze-thaw cycles at -80°C. CoolCutter™ SUMO protease is stable for at least 12 months at –80°C. Store 10× SUMO protease buffer at 4°C or –20°C.

## ■ 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, and delivers superior SUMO cleavage activity with both 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 CoolCutter™ SUMO Protease 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. In addition, the cleavage efficiency of CoolCutter™ SUMO Protease is low for 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.

## ■ Source

Recombinant *Saccharomyces Cerevisiae* ULP1 (Ubl-specific protease 1) gene expressed in E.coli.

## ■ Quality Control

Non-specific protease activity is undetectable.

## ■ Unite 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.

## ■ Composition

**SUMO Protease storage buffer :**

25 mM Tris-HCl, pH 8.0
1% Igepal (NP-40)
250 mM NaCl
50 μM DTT
50% (V / V) glycerol

**10× SUMO Protease Buffer (with salt) :**

500 mM Tris-HCl, pH 8.0
2% Igepal (NP-40)
1.5 M NaCl
10 mM DTT

**10× SUMO Protease Buffer (without salt) :**

500 mM Tris-HC, pH 8.0
2% Igepal (NP-40)
10 mM DTT

## ■ Suggested Reaction System

| Reagent | Volume | Final concentration |
|---|---|---|
| SUMO fusion protein | 5 μL | 10 μg |
| CoolCutter™ SUMO Protease | 1 μL | 2U |
| 10×SUMO Protease Buffer (with salt / without salt) | 10 μL | 1× |
| dd$H_2O$ | 84 μL | |
| Total volume | 100 μL | |

**Recommended incubation times at different temperatures:**

| Temperature | Time |
|---|---|
| 4°C | 15~16 h |
| 16°C | 4 h |
| 25°C | 1.5 h |
| 30°C | 1 h |

**Guidelines for Cleavage**

1. For most fusion protein, the suggested NaCl concentration in reaction mixture is 150mM; however, conditions may be optimized by varying the NaCl concentration from 100mM to 300mM. When setting up your cleavage reaction, use the apropriate 10× SUMO protease buffer(with salt / without salt).
2. Take into account the imidazole concentration in the reaction mixture, when imidazole concentration higher than 150mM, it will adversely affect the activity of the protease.
3. The percent of the substrate cleavage by the protease can be optimized by adjusting the incubation time and reaction temperature. If time is critical, add more protease to increase hydrolysis.