

SUMO-1 [6His-tagged]

Modifying Protein

Alternate Names: SMT3C, SMT3H3, UBL1, PIC1, GMP1, OFC10 and sentrin

Cat. No. 60-0002-500

Lot. No. 1364

Quantity: 500 µg

Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 1 of 2

Background

Small Ubiquitin-like Modifiers (SUMOs) are a family of small, related proteins that can be enzymatically conjugated to a target protein by a post-translational modification process termed SUMOylation. SUMO-1 is a highly conserved, small ubiquitin-related modifier that has been shown to be covalently conjugated to a large variety of cellular proteins (Kamitani *et al.*, 1997; Mahajan *et al.*, 1997; Matunis *et al.*, 1996). Cloning of SUMO-1 was first described by Boddy *et al.* (1996). SUMO-2 and SUMO-3 share 95% sequence identity, but only 50% sequence identity to SUMO-1. SUMO-1 is conjugated to a target protein in a similar way to ubiquitin and has been implicated in multiple cellular processes, including nuclear transport, cell cycle control, oncogenesis, inflammation and response to viral infection. SUMO-1 conjugation forms an isopeptide bond between Gly97 at the C-terminus of SUMO-1 and the ε-amino group on the Lysine side chain of the target protein; however it is unable to form multi-chain species (Bayer *et al.*, 1998; Mahajan *et al.*, 1997; Mahajan *et al.*, 1998). SUMO-1 targets substrates including RanGAP1, PML, Sp100, HSF1, Smad4, IκBα, c-Jun, p53 and Mdm2 (Melchior, 2000). RanGAP1, a Ran GTPase-activating is a major SUMO-1 substrate protein involved in nucleocytoplasmic trafficking (Swaminathan *et al.*, 2004). SUMO-1 covalently modifies a single lysine residue at position 526 in the C-terminus of RanGAP1 (Mahajan *et al.*, 1997; Matunis *et al.*, 1996;

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Physical Characteristics

Species: human

Source: *E. coli* expression

Quantity: 500 µg

Concentration: 1 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~13.5 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70°C; aliquot as required

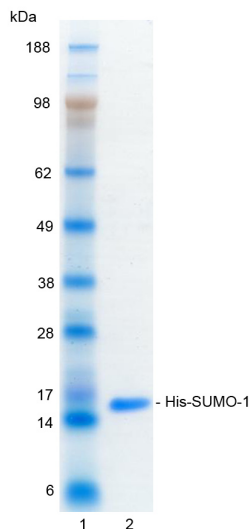
Protein Sequence:

MGSSHHHHHSSGLEVLFGQPGSMDSQEAKPST
EDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTH
LKKLKEYCQRQGVPMNSLRFLFEGQRIADNHTP
KELGMEEDVIEVYQEQTGG

Tag (**bold text**): N-terminal His
Protease cleavage site: PreScission™ (LEVLFGQ▼GP)
SUMO-1 (regular text): Start **bold italics** (amino acid residues 1-97)
Accession number: NP_003343

Quality Assurance

Purity: 4-12% gradient SDS-PAGE InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-SUMO-1



Protein Identification:

Confirmed by mass spectrometry.

E2 Thioester SUMO-1 Loading Assay:

The activity of His-SUMO-1 was validated by loading His-SUMO-1 onto the active cysteine of the UBE2I E2 enzyme via a transthioylation reaction. Incubation of His-SUMO-1, SAE1/SAE2 and UBE2I enzymes in the presence of ATP at 30°C was compared at two time points, T₀ and T₁₀ minutes. Sensitivity of the His-SUMO-1/UBE2I thioester bond to the reducing agent DTT was confirmed.



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Lot-specific COA version tracker: v1.0.0

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Background

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Muller *et al.*, 1998). SUMO-1 modified RanGAP1 has been found tightly associated with the nuclear envelope (Mahajan *et al.*, 1997; Matunis *et al.*, 1996) an observation which supports its role in nucleocytoplasmic trafficking.

References:

Bayer P, Arndt A, Metzger S, Mahajan R, Melchior F, Jaenicke R, Becker J (1998) Structure determination of the small ubiquitin-related modifier SUMO-1. *J Mol Biol* **280**, 275-86.

Boddy MN, Howe K, Etkin LD, Solomon E, Freemont PS (1996) PIC 1, a novel ubiquitin-like protein which interacts with the PML component of a multiprotein complex that is disrupted in acute promyelocytic leukaemia. *Oncogene* **13**, 971-82.

Kamitani T, Nguyen HP, Yeh ET (1997) Preferential modification of nuclear proteins by a novel ubiquitin-like molecule. *J Biol Chem* **272**, 14001-4.

Mahajan R, Delphin C, Guan T, Gerace L, Melchior F (1997) A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. *Cell* **88**, 97-107.

Mahajan R, Gerace L, Melchior F (1998) Molecular characterization of the SUMO-1 modification of RanGAP1 and its role in nuclear envelope association. *J Cell Biol* **140**, 259-70.

Matunis MJ, Coutavas E, Blobel G (1996) A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. *J Cell Biol* **135**, 1457-70.

Melchior F (2000) SUMO--nonclassical ubiquitin. *Annu Rev Cell Dev Biol* **16**, 591-626.



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