SUMO-1 [untagged]

Modifying Protein

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

Cat. No. 60-0006-500 Quantity: 500 µg -70°C Lot. No. 1854 Storage:

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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, IkBa, 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: ~11.1 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

aliquot as required

Protein Sequence:

G P G S M S D Q E A K P S T E D L G D K K E G EYIKLKVIGQDSSEIHFKVKMTTHLKKLKESY CQRQGVPMNSLRFLFEGQRIADNHTPKELG MEEEDVIEVYQEQTGG

The residues <u>underlined</u> remain after cleavage and removal of the purification tag

SUMO-1 (regular text): Start bold italics (amino acid

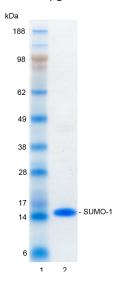
residues 1-97)

Accession number: NP_003343

Quality Assurance

Purity:

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



Protein Identification:

Confirmed by mass spectrometry.

E2 Thioester SUMO-1 Loading Assay:

The activity of SUMO-1 was validated by loading SUMO-1 onto the active cysteine of the UBE2I E2 enzyme via a transthiolation reaction. Incubation of SUMO-1, SAE1/SAE2 and UBE2I enzymes in the presence of ATP at 30 $^{\circ}$ C was compared at two time points, T $_{_{0}}$ and T₁₀ minutes. Sensitivity of the 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

Continued from page 1

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 Ran-GAP1 to nuclear pore complex protein RanBP2. Cell 88, 97-

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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