

SMURF1 [GST-tagged]

E3 Ligase

Alternate Names: E3 ubiquitin ligase SMURF1; Smad ubiquitination regulatory factor 1; KIAA1625

Cat. No. 63-0027-025
Lot. No. 30028

Quantity: 25 µg
Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 1 of 2

Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including the regulated and targeted proteasome-dependent degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). Smad-Specific E3 Ubiquitin Protein Ligase 1 (SMURF1) is a member of the E3 protein ligase family and cloning of the human gene was first described by Zhu *et al.* (1999). SMURF1 is a HECT domain ubiquitin E3 ligase that has been shown to regulate the cell polarity and protrusive activity and motility of tumour cells. Atypical protein kinase C-zeta (PKCζ), an effector of the Cdc42/Rac1-PAR6 polarity complex, recruits SMURF1 to cellular protrusions where it controls the local level of Rho A through degradation of the Rho A in the lamellipodia and filopodia of the cell (Wang *et al.*, 2003). SMURF1 is also a negative regulator of the Bone Morphogenetic Protein (BMP) signalling pathway mediating the ubiquitylation and degradation of SMAD1 and SMAD5 (Zhu *et al.*, 1999). More recently SMURF1 has been shown to ubiquitylate and degrade Krüppel-like factor 2 (KLF2) a transcription factor essential for normal lung development (Xie *et al.*, 2011).

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Physical Characteristics

Species: human

Protein Sequence: Please see page 2

Source: *E. coli* expression

Quantity: 25 µg

Concentration: 0.5 mg/ml

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

Molecular Weight: ~114 kDa

Purity: >90% by InstantBlue™ SDS-PAGE

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

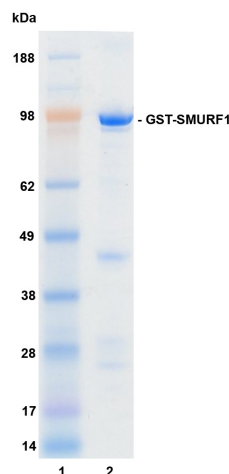
Quality Assurance

Protein Identification:

Confirmed by mass spectrometry.

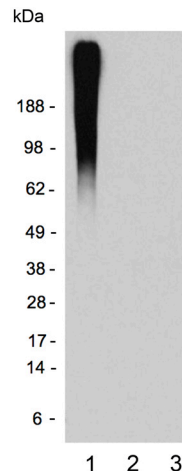
Purity:

4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-SMURF1



E3 ligase assay:

The ubiquitin conjugating activity of GST-SMURF1 was validated through its ability to catalyse the generation of polyubiquitin chains in the presence of the E1 activating enzyme His-UBE1, the E2 conjugating enzyme His-UBE2D2 (UbcH5b) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of GST-SMURF1 for 30 minutes at 30°C in the presence of ubiquitin, His-UBE1, His-UBE2D2 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or GST-SMURF1 (Lane 3) excluded from the reaction. Ubiquitin conjugates were identified by Western blotting using an anti-ubiquitin conjugate antibody and these were observed only in the presence of both ATP and GST-SMURF1.



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Lot-specific COA version tracker: v1.0.1

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Background

Continued from page 1

References:

Wang HR, Zhang Y, Ozdamar B, Ogunjimi AA, Alexandrova E, Thomsen GH, Wrana JL (2003) Regulation of cell polarity and protrusion formation by targeting RhoA for degradation. *Science* **302**, 1775-9.

Xie P, Tang Y, Shen S, Wang Y, Xing G, Yin Y, He F, Zhang L (2011) Smurf1 ubiquitin ligase targets Kruppel-like factor KLF2 for ubiquitination and degradation in human lung cancer H1299 cells. *Biochem Biophys Res Commun* **407**, 254-9.

Zhu H, Kavsak P, Abdollah S, Wrana JL, Thomsen GH (1999) A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. *Nature* **400**, 687-93.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTSMAIIRYIADKHN
MLGGCPKERAEISMLEGAVLDIRYGV
RIAYSKDFETLKVDFLSKLP EMLKMF
DRLCHKTYLNGDHVTHPDFMFLYDALDV
VLYMDPMCLDAFPKLVCFKKRIEAIPO
IDKYLKSSKYIAWPLQGWQATFGGGDHP
PKSDLEVLFGGPLGSPEIPGSTRAAAM
SNPGTRRNGSSIKIRLTVLCAKNLAKKDF
FRLPDPFAKIVVDGSGQCHSTDTVKNLTD
PKWNQHYDLYVGKTDSTITISVWNHKKIHK
KQGAGFLGCVRLLSNAISRKDTGYQRLD
LCKLNPSTDAVRGQIVVSLQTRDRIGT
GGSVVDCRGLLENEGTVYEDSGPGRPLSCF
MEEPAPYTDSTGAAAGGGNCRFVES P
SQDQRLQAQRLRNPDVRGSLQTPQNRPH
GHQSPPELPEGYEQRTTVQGQVYFLHTQT
GVSTWHDPRI PPSG T I PGGDAAFLYE
FLLQGHTSEPRDLNSVNCDELGLPLPPGW
EVRSTVSGRIYFVDHNNRRTTQFTDPR LH
HIMNHQCQLKEPSQPLPLPSEGSLEDEEL
PAQRYERDLVQKLVLRHEL SLQPPQAGH
CRIEVSREEIFEESYRQIMKMRPKDLK
KRLMVKFRGEEGLDYGGVAREWLYLLCHEM
LNPYYGLFQYSTDN IYMLQINPDSSIN
PDHLSYFHFVGRIMGLAVFHGHYING
GFTVPFYKQLLGKPIQLSDLESVDPEL
HKS LVWILENDITPVL DHTFCVEHNAF
GRILQHELKPNGRNVPVTEENKKEYVRLY
VNWRFMRGIEAQFLALQKGFNELIPQHLLK
PFDQKELELIIGGLDKIDLNDWKS N
TRLKHCVADSNIVRWFVQAVETTFDEER
RARLLQFVTGSTRVPLQGFKALQGST
GAAGPRLFTIHLIDANTDNLPKAHTCFNRID
IPPYESYEKLYEKLLTAVEETCGFAV

Tag (**bold text**): N-terminal GST

Protease cleavage site: PreScission™ (LEVLFG▼GP)

SMURF1 (regular text): Start **bold italics** (amino acid residues 1-757)

Accession number: NP_065162.1



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