

# 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).

Continued on page 2

## 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

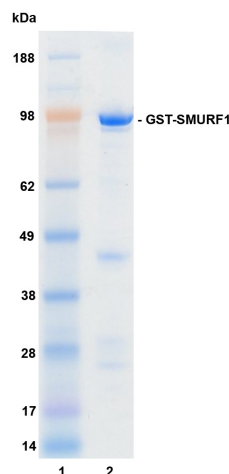
## Quality Assurance

### Protein Identification:

Confirmed by mass spectrometry.

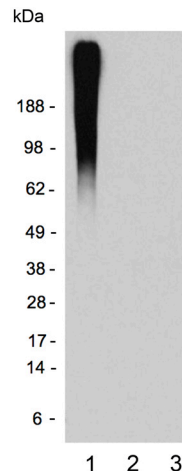
### Purity:

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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## 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:

**M**S P I L G Y W K I K G L V Q P T R L L L E Y L E E K Y  
**E**E H L Y E R D E G D K W R N K K F E L G L E F P N  
L P Y Y I D G D V K L T Q S M A I I R Y I A D K H N  
M L G G C P K E R A E I S M L E G A V L D I R Y G V S  
R I A Y S K D F E T L K V D F L S K L P E M L K M F E  
D R L C H K T Y L N G D H V T H P D F M L Y D A L D V  
V L Y M D P M C L D A F P K L V C F K K R I E A I P Q  
I D K Y L K S S K Y I A W P L Q G W Q A T F G G G D H P  
P K S D **L E V L F Q G P** L G S P E I P G S T R A A A M  
S N P G T R R N G S S I K I R L T V L C A K N L A K K D F  
F R L P D P F A K I V V D G S G Q C H S T D T V K N T L D  
P K W N Q H Y D L Y V G K T D S I T I S V W N H K K I H K  
K Q G A G F L G C V R L L S N A I S R L K D T G Y Q R L D  
L C K L N P S D T D A V R G Q I V V S L Q T R D R I G T  
G G S V V D C R G L L E N E G T V Y E D S G P G R P L S C F  
M E E P A P Y T D S T G A A A G G G N C R F V E S P  
S Q D Q R L Q A Q R L R N P D V R G S L Q T P Q N R P H  
G H Q S P E L P E G Y E Q R T T V Q G Q V Y F L H T Q T  
G V S T W H D P R I P S P S G T I P G G D A A F L Y E  
F L L Q G H T S E P R D L N S V N C D E L G P L P P G W  
E V R S T V S G R I Y F V D H N N R T T Q F T D P R L H  
H I M N H Q C Q L K E P S Q P L P L P S E G S L E D E E L  
P A Q R Y E R D L V Q K L K V L R H E L S L Q Q P Q A G H  
C R I E V S R E E I F E E S Y R Q I M K M R P K D L K  
K R L M V K F R G E E G L D Y G G V A R E W L Y L L C H E M  
L N P Y Y G L F Q Y S T D N I Y M L Q I N P D S S I N  
P D H L S Y F H F V G R I M G L A V F H G H Y I N G  
G F T V P F Y K Q L L G K P I Q L S D L E S V D P E L  
H K S L V W I L E N D I T P V L D H T F C V E H N A F  
G R I L Q H E L K P N G R N V P V T E E N K K E Y V R L Y  
V N W R F M R G I E A Q F L A L Q K G F N E L I P Q H L L K  
P F D Q K E L E L I I G G L D K I D L N D W K S N  
T R L K H C V A D S N I V R W F W Q A V E T F D E E R  
R A R L L Q F V T G S T R V P L Q G F K A L Q G S T  
G A A G P R L F T I H L I D A N T D N L P K A H T C F N R I D  
I P P Y E S Y E K L Y E K L L T A V E E T C G F A V

Tag (**bold text**): N-terminal GST  
Protease cleavage site: PreScission™ (**L E V L F Q ▼ G P**)  
SMURF1 (regular text): Start **bold italics** (amino acid residues 1-757)  
Accession number: NP\_065162.1



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