SMURF2 [GST-tagged]

E3 Ligase

Alternate Names: SMAD specific E3 ubiquitin protein ligase 2, MGC138150

Cat. No.	**63-0046-025**	Quantity:	25 µg
Lot. No.	**30231**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

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 (SMURF2) is a member of the E3 protein ligase family and cloning of the human gene was first described by Kavsak *et al.* (2000). SMURF2 is a HECT domain ubiquitin E3 ligase that has been shown to regulate cell polarity, senescence and tumor suppression (Blank *et al.*, 2012). Immunoprecipitation studies have demonstrated that SMURF2 interacts with RNF11 through the binding of the WW domain 2 and 3 of SMURF2 to the PY motif of RNF11. RNF11 was also found to interact with Ube2D1 in this complex and ubiquitylation of both SMURF2 and RNF11 was detected. (Subramaniam *et al.*, 2003). Knock down of SMURF2 in human tumour cell lines results in increased levels of RNF20 and ubiquitylation of the RNF20 substrate histone H2B (Blank et al., 2012). SMURF2 knockout mice appear normal until early adulthood, when a large number of them develop tumours of all types (Blank *et al.*, 2012).

References:

Blank M, Tang Y, Yamashita M, Burkett SS, Cheng SY *et al.* (2012) A tumor suppressor function of Smurf2 associated with controlling chromatin landscape and genome stability through RNF20. *Nature Med* **18**, 227-234.

Continued on page 2

Physical Characteristics

Species: human

Source: *E. coli*

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

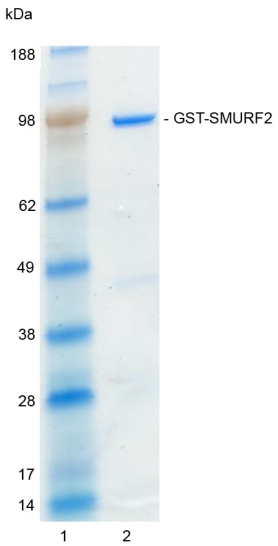
Purity: >90% by InstantBlue™ SDS-PAGE

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

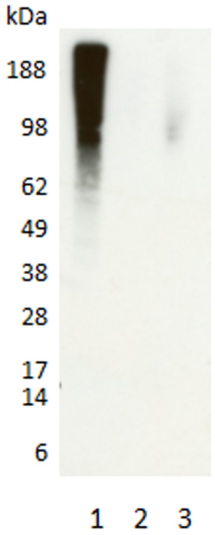
Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-SMURF2



Protein Identification:
Confirmed by mass spectrometry.



E3 ligase assay: The ubiquitin conjugating activity of GST-SMURF2 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-UBE2D3 (UbcH5c) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of GST-SMURF2 for 60 minutes at 30˚C in the presence of ubiquitin, His-UBE1, His-UBE2D3 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or GST-SMURF2 (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-SMURF2.

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0020
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

SMURF2 [GST-tagged]

E3 Ligase

Alternate Names: SMAD specific E3 ubiquitin protein ligase 2, MGC138150

Cat. No.	**63-0046-025**	Quantity:	25 µg
Lot. No.	**30231**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

Background

Continued from page 1

Kavsak P, Rasmussen RK, Causing CG, Bonni S *et al.* (2000) Smad7 binds to Smurf2 to form an E3 ubiquitin ligase that targets the TGF-beta receptor for degradation. *Molec Cell* **6**, 1365-1375.

Subramaniam V, Li H, Wong M, Kitching R, Attisano L, Wrana J, Zubovits J, Burger AM, Seth A (2003) The RING-H2 protein RNF11 is overexpressed in breast cancer and is a target of Smurf2 E3 ligase. *Brit J Cancer* **89**, 1538-1544.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTQSMAIIRYIADKHNMLG
GCPKERAEISMLEGAVLDIRYGVSRIAY
SKDFETLKVDFLSKLPEMLKMFEDRLCHK
TYLNGDHVTHPDFMLYDALDVVLYMDPM
CLDAFPKLVCFKKRIEAIPQIDKYLKSSKY
IAWPLQGWQATFGGGDHPPKSDLEVLFQG
PLGSPEIPGSTRAAA***M***SNPGRRRNGPVKLR
LTVLCAKNLVKKDFFRLPDPFAKVVVDGS
GQCHSTDTVKNTLDPKWNQHYDLYIGKSDS
VTISVWNHKKIHKKQGAGFLGCVRLLSNAIN
RLKDTGYQRLDLCKLGPNDNDTVRGQIVVS
LQSRDRIGTGGQVVDCSRLFDNDLPDGWEER
RTASGRIQYLNHITRTTQWERPTRPASEY
SSPGRPLSCFVDENTPISGTNGATCGQSS
DPRLAERRVRSQRHRNYMSRTHLHTPP
DLPEGYEQRTTQQGQVYFLHTQTGVSTWH
DPRVPRDLSNINCEELGPLPPGWEIRN
TATGRVYFVDHNNRTTQFTDPRLSANL
HLVLNRQNQLKDQQQQQVVSLCPDDTE
CLTVPRYKRDLVQKLKILRQELSQQQPQAGH
CRIEVSREEIFEESYRQVMKMRPKDLWKRL
MIKFRGEEGLDYGGVAREWLYLLSHEMLN
PYYGLFQYSRDDIYTLQINPDSAVNPEHLSY
FHFVGRIMGMAVFHGHYIDGGFTLPFYKQLL
GKSITLDDMELVDPDLHNSLVWILENDIT
GVLDHTFCVEHNAYGEIIQHELKPNGK
SIPVNEENKKEYVRLYVNWRFLRGIEAQFLA
LQKGFNEVIPQHLLKTFDEKELELIICGLG
KIDVNDWKVNTRLKHCTPDSNIVKWFWKAV
EFFDEERRARLLQFVTGSSRVPLQGFKALQ
GAAGPRLFTIHQIDACTNNLPKAHTCFNRID
IPPYESYEKLYEKLLTAIEETCGFAVE

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
SMURF2 (regular text): Start ***bold italics*** (amino acid residues 1-748)
Accession number: AAG45422.1

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0020
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0