Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-Smurf1

Enzyme description:- GST-Smurf1 1-754 (full length)

Clone number:- DU19628

Source:- Recombinant

Tag:- N-terminal GST

Purification method:- GSH-Sepharose

Expression level:- 0.1mg/L

Calculated molecular mass:-

Monoisotopic 113916
Average Mass 113986
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.71

Purity:- 80%

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT

Storage temperature:- -80ºC

Assay:-

Ligase assay with Ubiquitin, UBE1 and UBE2D family members. Incubate with 0.5mM Mg-ATP for 2h at 37ºC and analyse by immunoblotting for Ubiquitin chain formation.

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Clone Data Sheet

Protein name

Protein	Smurf1
Synonyms	Smuf1
Clone Number	DU19628
Species	Human
Accession Number	Q9HCE7
Tags	N-terminal GST
Aminoacid sequence of the expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEIPGSTRAAA**MSN** **PGTRRNGSSIKIRLTVLCAKNLAKKDFFRLPDPFAKIVVDGSGQCHSTD** **TVKNTLDPKWNQHYDLYVGKTDSITISVWNHKKIHKKQGAGFLGCVRLL** **SNAISRLKDTGYQRLDLCKLNPSDTDAVRGQIVVSLQTRDRIGTGGSVV** **DCRGLLENEGTVYEDSGPGRPLSCFMEEPAPYTDSTGAAAGGGNCRFVE** **SPSQDQRLQAQRLRNPDVRGSLQTPQNRPHGHQSPELPEGYEQRTTVQG** **QVYFLHTQTGVSTWHDPRIPSPSGTIPGGDAAFLYEFLLQGHTSEPRDL** **NSVNCDELGPLPPGWEVRSTVSGRIYFVDHNNRTTQFTDPRLHHIMNHQ** **CQLKEPSQPLPLPSEGSLEDEELPAQRYERDLVQKLKVLRHELSLQQPQ** **AGHCRIEVSREEIFEESYRQIMKMRPKDLKKRLMVKFRGEEGLDYGGVA** **REWLYLLCHEMLNPYYGLFQYSTDNIYMLQINPDSSINPDHLSYFHFVG** **RIMGLAVFHGHYINGGFTVPFYKQLLGKPIQLSDLESVDPELHKSLVWI** **LENDITPVLDHTFCVEHNAFGRILQHELKPNGRNVPVTEENKKEYVRLY** **VNWRFMRGIEAQFLALQKGFNELIPQHLLKPFDQKELELIIGGLDKIDL** **NDWKSNTRLKHCVADSNIVRWFWQAVETFDEERRARLLQFVTGSTRVPL** **QGFKALQGSTGAAGPRLFTIHLIDANTDNLPKAHTCFNRIDIPPYESYE** **KLYEKLLTAVEETCGFAVE**
Native sequence	in bold
Protease cleavage	Prescission protease site underlined
Cloning sites	Not1 / Not1

DNA sequence of insert

GCGGCCGCTATGTCGAACCCCGGGACACGCAGGAACGGCTCCAGCATCA
AGATCCGTCTGACAGTGTTATGTGCCAAGAACCTTGCAAAGAAAGACTT
CTTCAGGCTCCCTGACCCTTTTGCAAAGATTGTCGTGGATGGGTCTGGG
CAGTGCCACTCAACCGACACTGTGAAAAACACATTGGACCCAAAGTGGA
ACCAGCACTATGATCTATATGTTGGGAAAACGGATTCGATAACCATTAG
CGTGTGGAACCATAAGAAAATTCACAAGAAACAGGGAGCTGGCTTCCTG
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ACCGGCGGCTCGGTGGTGGACTGCAGAGGACTGTTAGAAAATGAAGGAA
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CCATATGAGTCCTATGAGAAGCTCTACGAGAAGCTGCTGACAGCCGTGG
AGGAGACCTGCGGGTTTGCTGTGGAGTGAGCGGCCGC