Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of SMAD6 [1 – 496]

Enzyme description:- SMAD6 [1 – 496]

Clone number:- DU 12813

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 80, 270.46 daltons
Average Mass 80, 321.41 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 7.16

Purity:- >80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

SMAD6 [1 – 496]

Protein	SMAD6 [1 – 496]
Clone number	DU 12813
Species	Human
Accession number	O43541.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MFRSKRSGLVRRLW** **RSRVVPDREEGGSGGGGGGDEDGSLGSRAEPAPRAREGGGCGRSEVRPV** **APRRPRDAVGQRGAQGAGRRRRAGGPPRPMSEPGAGAGSSLLDVAEPGG** **PGWLPESDCETVTCCLFSERDAAGAPRDASDPLAGAALEPAGGGRSREA** **RSRLLLLEQELKTVTYSLLKRLKERSLDTLLEAVESRGGVPGGCVLVPR** **ADLRLGGQPAPPQLLLGRLFRWPDLQHAVELKPLCGCHSFAAAADGPTV** **CCNPYHFSRLCGPESPPPPYSRLSPRDEYKPLDLSDSTLSYTETEATNS** **LITAPGEFSDASMSPDATKPSHWCSVAYWEHRTRVGRLYAVYDQAVSIF** **YDLPQGSGFCLGQLNLEQRSESVRRTRSKIGFGILLSKEPDGVWAYNRG** **EHPIFVNSPTLDAPGGRALVVRKVPPGYSIKVFDFERSGLQHAPEPDAA** **DGPYDPNSVRISFAKGWGPCYSRQFITSCPCWLEILLNNPR**
Native sequence	Amino acids M1 – R496 (end) of human SMAD6. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*H1 sites of pGEX6P-1

Nucleotide Sequence of insert

ggatccATGTTCAGGTCCAAACGCTCGGGGCTGGTGCGGCGACTTTGGC
GAAGTCGTGTGGTCCCCGACCGGGAGGAAGGCGGCAGCGGCGGCGGCGG
TGGCGGCGACGAGGATGGGAGCTTGGGCAGCCGAGCTGAGCCGGCCCCG
CGGGCAAGAGAGGGCGGAGGCTGCGGCCGCTCCGAAGTCCGCCCGGTAG
CCCCGCGGCGGCCCCGGGACGCAGTGGGACAGCGAGGCGCCCAGGGCGC
GGGGAGGCGCCGGCGCGCAGGGGGCCCCCCGAGGCCCATGTCGGAGCCA
GGGGCCGGCGCTGGGAGCTCCCTGCTGGACGTGGCGGAGCCGGGAGGCC
CGGGCTGGCTGCCCGAGAGTGACTGCGAGACGGTGACCTGCTGTCTCTT
TTCGGAGCGGGACGCCGCCGGCGCGCCCCGGGACGCCAGCGACCCCCTG
GCCGGGGCGGCCCTGGAGCCGGCGGGCGGCGGGCGGAGTCGCGAAGCGC
GCTCGCGGCTGCTGCTGCTGGAGCAGGAACTCAAAACCGTCACGTACTC
GCTGCTGAAGCGGCTCAAGGAGCGCTCGCTGGACACGCTGCTGGAGGCG
GTGGAGTCCCGCGGCGGCGTGCCGGGCGGCTGCGTGCTGGTGCCGCGCG
CCGACCTCCGCCTGGGCGGCCAGCCCGCGCCGCCGCAGCTGCTGCTCGG
CCGCCTCTTTCGCTGGCCCGACCTGCAGCACGCCGTGGAGCTGAAGCCC
CTGTGCGGCTGCCACAGCTTCGCCGCCGCCGCCGACGGCCCTACCGTGT
GCTGCAACCCCTACCACTTCAGCCGGCTCTGCGGGCCCGAATCTCCGCC
ACCTCCCTACTCTCGGCTGTCTCCTCGCGACGAGTACAAGCCACTGGAT
CTGTCCGATTCCACATTGTCTTACACTGAAACGGAGGCTACCAACTCCC
TCATCACTGCTCCGGGTGAATTCTCAGACGCCAGCATGTCTCCGGACGC
CACCAAGCCGAGCCACTGGTGCAGCGTGGCGTACTGGGAGCACCGGACG
CGCGTGGGCCGCCTCTATGCGGTGTACGACCAGGCCGTCAGCATCTTCT
ACGACCTACCTCAGGGCAGCGGCTTCTGCCTGGGCCAGCTCAACCTGGA
GCAGCGCAGCGAGTCGGTGCGGCGAACGCGCAGCAAGATCGGCTTCGGC
ATCCTGCTCAGCAAGGAGCCCGACGGCGTGTGGGCCTACAACCGCGGCG
AGCACCCCATCTTCGTCAACTCCCCGACGCTGGACGCGCCCGGCGGCCG
CGCCCTGGTCGTGCGCAAGGTGCCCCCCGGCTACTCCATCAAGGTGTTC
GACTTCGAGCGCTCGGGCCTGCAGCACGCGCCCGAGCCCGACGCCGCCG
ACGGCCCCTACGACCCCAACAGCGTCCGCATCAGCTTCGCCAAGGGCTG
GGGGCCCTGCTACTCCCGGCAGTTCATCACCTCCTGCCCCTGCTGGCTG
GAGATCCTCCTCAACAACCCCAGAtagggatcc