Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of SMAD1 [1 – 465]

Enzyme description:- SMAD1 [1 – 465]

Clone number:- DU 19269

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 79, 033.12 daltons
Average Mass 79, 084.20 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.49

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

SMAD1 [1 – 465]

Protein	SMAD1 [1 – 465]
Clone number	DU 19269
Species	Human
Accession number	NM_005900.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MNVTSLFSFTSPAV** **KRLLGWKQGDEEEKWAEKAVDALVKKLKKKKGAMEELEKALSCPGQPSN** **CVTIPRSLDGRLQVSHRKGLPHVIYCRVWRWPDLQSHHELKPLECCEFP** **FGSKQKEVCINPYHYKRVESPVLPPVLVPRHSEYNPQHSLLAQFRNLGQ** **NEPHMPLNATFPDSFQQPNSHPFPHSPNSSYPNSPGSSSSTYPHSPTSS** **DPGSPFQMPADTPPPAYLPPEDPMTQDGSQPMDTNMMAPPLPSEINRGD** **VQAVAYEEPKHWCSIVYYELNNRVGEAFHASSTSVLVDGFTDPSNNKNR** **FCLGLLSNVNRNSTIENTRRHIGKGVHLYYVGGEVYAECLSDSSIFVQS** **RNCNYHHGFHPTTVCKIPSGCSLKIFNNQEFAQLLAQSVNHGFETVYEL** **TKMCTIRMSFVKGWGAEYHRQDVTSTPCWIEIHLHGPLQWLDKVLTQMG** **SPHNPISSVS**
Native sequence	Amino acids M1 – S465 (end) of human SMAD1. 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 and *Not*1 sites of pGEX6P-1

Nucleotide Sequence of insert

ggatccATGAATGTGACAAGTTTATTTTCCTTTACAAGTCCAGCTGTGA
AGAGACTTCTTGGGTGGAAACAGGGCGATGAAGAAGAAAAATGGGCAGA
GAAAGCTGTTGATGCTTTGGTGAAAAAACTGAAGAAAAAGAAAGGTGCC
ATGGAGGAACTGGAAAAGGCCTTGAGCTGCCCAGGGCAACCGAGTAACT
GTGTCACCATTCCCCGCTCTCTGGATGGCAGGCTGCAAGTCTCCCACCG
GAAGGGACTGCCTCATGTCATTTACTGCCGTGTGTGGCGCTGGCCCGAT
CTTCAGAGCCACCATGAACTAAAACCACTGGAATGCTGTGAGTTTCCTT
TTGGTTCCAAGCAGAAGGAGGTCTGCATCAATCCCTACCACTATAAGAG
AGTAGAAAGCCCTGTACTTCCTCCTGTGCTGGTTCCAAGACACAGCGAA
TATAATCCTCAGCACAGCCTCTTAGCTCAGTTCCGTAACTTAGGACAAA
ATGAGCCTCACATGCCACTCAACGCCACTTTTCCAGATTCTTTCCAGCA
ACCCAACAGCCACCCGTTTCCTCACTCTCCCAATAGCAGTTACCCAAAC
TCTCCTGGGAGCAGCAGCAGCACCTACCCTCACTCTCCCACCAGCTCAG
ACCCAGGAAGCCCTTTCCAGATGCCAGCTGATACGCCCCCACCTGCTTA
CCTGCCTCCTGAAGACCCCATGACCCAGGATGGCTCTCAGCCGATGGAC
ACAAACATGATGGCGCCTCCCCTGCCCTCAGAAATCAACAGAGGAGATG
TTCAGGCGGTTGCTTATGAGGAACCAAAACACTGGTGCTCTATTGTCTA
CTATGAGCTCAACAATCGTGTGGGTGAAGCGTTCCATGCCTCCTCCACA
AGTGTGTTGGTGGATGGTTTCACTGATCCTTCCAACAATAAGAACCGTT
TCTGCCTTGGGCTGCTCTCCAATGTTAACCGGAATTCCACTATTGAAAA
CACCAGGCGGCATATTGGAAAAGGAGTTCATCTTTATTATGTTGGAGGG
GAGGTGTATGCCGAATGCCTTAGTGACAGTAGCATCTTTGTGCAAAGTC
GGAACTGCAACTACCATCATGGATTTCATCCTACTACTGTTTGCAAGAT
CCCTAGTGGGTGTAGTCTGAAAATTTTTAACAACCAAGAATTTGCTCAG
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CAAAAATGTGTACTATACGTATGAGCTTTGTGAAGGGCTGGGGAGCAGA
ATACCACCGCCAGGATGTTACTAGCACCCCCTGCTGGATTGAGATACAT
CTGCACGGCCCCCTCCAGTGGCTGGATAAAGTTCTTACTCAAATGGGTT
CACCTCATAATCCTATTTCATCTGTATCTtaagcggccgc