Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of SMAD2 [1 – 467]

Enzyme description:- SMAD2 [1 - 467]

Clone number:- DU 19395

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 79, 452.57 daltons
Average Mass 79, 503.70 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.93

Purity:- >80 %

Activation protocol:- Constitutively active

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

Assay:- Substrate for TGFBR1

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Clone Data Sheet

SMAD2 [1 – 467]

Protein	SMAD2 [1 - 467]
Clone number	DU 19395
Species	Human
Accession number	NM_005901
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**MSSILPFTPPV** **VKRLLGWKKSAGGSGGAGGGEQNGQEEKWCEKAVKSLVKKLKKTGRLDE** **LEKAITTQNCNTKCVTIPSTCSEIWGLSTPNTIDQWDTTGLYSFSEQTR** **SLDGRLQVSHRKGLPHVIYCRLWRWPDLHSHHELKAIENCEYAFNLKKD** **EVCVNPYHYQRVETPVLPPVLVPRHTEILTELPPLDDYTHSIPENTNFP** **AGIEPQSNYIPETPPPGYISEDGETSDQQLNQSMDTGSPAELSPTTLSP** **VNHSLDLQPVTYSEPAFWCSIAYYELNQRVGETFHASQPSLTVDGFTDP** **SNSERFCLGLLSNVNRNATVEMTRRHIGRGVRLYYIGGEVFAECLSDSA** **IFVQSPNCNQRYGWHPATVCKIPPGCNLKIFNNQEFAALLAQSVNQGFE** **AVYQLTRMCTIRMSFVKGWGAEYRRQTVTSTPCWIELHLNGPLQWLDKV** **LTQMGSPSVRCSSMS**
Native sequence	Amino acids M1 – S425 (end) of human SMAD2. Residue M235 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 - 229
Cloning sites	*Eco*R1 and *Not*1sites of pGEX 6P-1

Nucleotide Sequence of insert

gaattcATGTCGTCCATCTTGCCATTCACGCCGCCAGTTGTG
AAGAGACTGCTGGGATGGAAGAAGTCAGCTGGTGGGTCTGGA
GGAGCAGGCGGAGGAGAGCAGAATGGGCAGGAAGAAAAGTGG
TGTGAGAAAGCAGTGAAAAGTCTGGTGAAGAAGCTAAAGAAA
ACAGGACGATTAGATGAGCTTGAGAAAGCCATCACCACTCAA
AACTGTAATACTAAATGTGTTACCATACCAAGCACTTGCTCT
GAAATTTGGGGACTGAGTACACCAAATACGATAGATCAGTGG
GATACAACAGGCCTTTACAGCTTCTCTGAACAAACCAGGTCT
CTTGATGGTCGTCTCCAGGTATCCCATCGAAAAGGATTGCCA
CATGTTATATATTGCCGATTATGGCGCTGGCCTGATCTTCAC
AGTCATCATGAACTCAAGGCAATTGAAAACTGCGAATATGCT
TTTAATCTTAAAAAGGATGAAGTATGTGTAAACCCTTACCAC
TATCAGAGAGTTGAGACACCAGTTTTGCCTCCAGTATTAGTG
CCCCGACACACCGAGATCCTAACAGAACTTCCGCCTCTGGAT
GACTATACTCACTCCATTCCAGAAAACACTAACTTCCCAGCA
GGAATTGAGCCACAGAGTAATTATATTCCAGAAACGCCACCT
CCTGGATATATCAGTGAAGATGGAGAAACAAGTGACCAACAG
TTGAATCAAAGTATGGACACAGGCTCTCCAGCAGAACTATCT
CCTACTACTCTTTCCCCTGTTAATCATAGCTTGGATTTACAG
CCAGTTACTTACTCAGAACCTGCATTTTGGTGTTCGATAGCA
TATTATGAATTAAATCAGAGGGTTGGAGAAACCTTCCATGCA
TCACAGCCCTCACTCACTGTAGATGGCTTTACAGACCCATCA
AATTCAGAGAGGTTCTGCTTAGGTTTACTCTCCAATGTTAAC
CGAAATGCCACGGTAGAAATGACAAGAAGGCATATAGGAAGA
GGAGTGCGCTTATACTACATAGGTGGGGAAGTTTTTGCTGAG
TGCCTAAGTGATAGTGCAATCTTTGTGCAGAGCCCCAATTGT
AATCAGAGATATGGCTGGCACCCTGCAACAGTGTGTAAAATT
CCACCAGGCTGTAATCTGAAGATCTTCAACAACCAGGAATTT
GCTGCTCTTCTGGCTCAGTCTGTTAATCAGGGTTTTGAAGCC
GTCTATCAGCTAACTAGAATGTGCACCATAAGAATGAGTTTT
GTGAAAGGGTGGGGAGCAGAATACCGAAGGCAGACGGTAACA
AGTACTCCTTGCTGGATTGAACTTCATCTGAATGGACCTCTA
CAGTGGTTGGACAAAGTATTAACTCAGATGGGATCCCCTTCA
GTGCGTTGCTCAAGCATGTCAtaagcggccgc