

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of SMAD3 [1 – 425]

Enzyme description:- SMAD3 [1 - 425]

Clone number:- DU 33974

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 74, 856.39 daltons

Average Mass 74, 904.92 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.33

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

SMAD3 [1 – 425]

<u>Protein</u>	SMAD3 [1 - 425]
<u>Clone number</u>	DU 33974
<u>Species</u>	Human
<u>Accession number</u>	NM_005902.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEELHYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGPLGSMSSILPFTPPIVKR LLGWKKGEQNGQEEKWCEKAVKSLVKKLKKTGQLELEKAITTONVNTK CITIPRSLDGRLQVSHRKGLPHVIYCRLWRWPDLHSHHEL RAMELCEFA FNMKKDEV CVNPHYQ RVETPVLPVLPVPRHTEIPA EFPPLDDYSHSIP ENTNFPAGIEPQSNIPETPPPGYLS EDGETSDHQMNHSM DAGSPNLSPN PMSPAHNNDLQPVTYCEPAFWCSI SYEELNQRVGETFHASQPSMTVDG F TDPNSERFCLGLLSNVNRNA AVELTRRH IGRGVRLYYIGGEVFAECL SDSAIFVQSPNCNORYGWHPATVCKIPPGCNLKI FNNQEF AALLAQSVN QGF EAVYQLTRMCTIRMSFVKGWGAEYRRQTVTSTPCWIELHLNGPLQW LDKVL TQMGSPSIRCSSVS</p>
<u>Native sequence</u>	<p>Amino acids M1 – S425 (end) of human SMAD3. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pGEX 6P-1

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Nucleotide
Sequence of insert

ggatccATGTCGTCCATCCTGCCTTTCACTCCCCGATCGTG
AAGCGCTGCTGGGCTGGAAGAAGGGCGAGCAGAACGGGCAG
GAGGAGAAATGGTGCAGAGAAGGCGGTCAAGAGCCTGGTCAAG
AAACTCAAGAAGACGGGGCAGCTGGACGAGCTGGAGAAGGCC
ATCACCACGCAGAACGTCAACACCAAGTGCATCACCATCCCC
AGGTCCCTGGATGGCCGGTTGCAGGTGTCCCATCGGAAGGGG
CTCCCTCATGTTCATCTACTGCCGCCTGTGGCGATGGCCAGAC
CTGCACAGCCACCACGAGCTGCGGGCCATGGAGCTGTGTGAG
TTCGCCTTCAATATGAAGAAGGACGAGGTCTGCGTGAATCCC
TACCACTACCAGAGAGTAGAGACACCAGTTCTACCTCCTGTG
TTGGTGCCACGCCACACAGAGATCCCGGCCGAGTTCCCCCA
CTGGACGACTACAGCCATTCCATCCCCGAAAACACTAACTTC
CCCGCAGGCATCGAGCCCCAGAGCAATATTCCAGAGACCCCA
CCCCCTGGCTACCTGAGTGAAGATGGAGAAACCAGTGACCAC
CAGATGAACCACAGCATGGACGCAGGTTCTCCAAACCTATCC
CCGAATCCGATGTCCCCAGCACATAATAACTTGGACCTGCAG
CCAGTTACCTACTGCGAGCCGGCCTTCTGGTGCTCCATCTCC
TACTACGAGCTGAACCAGCGCGTCGGGGAGACATTCACGCC
TCGCAGCCATCCATGACTGTGGATGGCTTCACCGACCCCTCC
AATTCGGAGCGCTTCTGCCTAGGGCTGCTCTCCAATGTCAAC
AGGAATGCAGCAGTGGAGCTGACACGGAGACACATCGGAAGA
GGCGTGCGGCTCTACTACATCGGAGGGGAGGTCTTCGCAGAG
TGCCTCAGTGACAGCGCTATTTTTGTCCAGTCTCCAACTGT
AACCAGCGCTATGGCTGGCACCCGGCCACCGTCTGCAAGATC
CCACCAGGATGCAACCTGAAGATCTTCAACAACCAGGAGTTC
GCTGCCCTCCTGGCCCAGTCGGTCAACCAGGGCTTTGAGGCT
GTCTACCAGTTGACCCGAATGTGCACCATCCGCATGAGCTTC
GTCAAAGGCTGGGGAGCGGAGTACAGGAGACAGACTGTGACC
AGTACCCCTGCTGGATTGAGCTGCACCTGAATGGGCCTTTG
CAGTGGCTTGACAAGGTCCTCACCCAGATGGGCTCCCCAAGC
ATCCGCTGTTCCAGTGTGTCTtaggcggccgc