Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of STRAD alpha [1 - 431]

Enzyme description:- STRAD alpha [1 – 431]

Clone number:- DU 2122

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 76, 354.54 daltons
Average Mass 76, 403.52 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.88

Purity:- >80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

STRAD alpha [1 - 431]

Protein	STRAD alpha [1 - 431]
Clone number	DU 2122
Species	Human
Accession number	AF308302.1
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPGIPGSPEF ALGS**MSFLVSKPERIRRWVSEKFIVEGLRDLELFGEQPPGDTRRKTND** **ASSESIASFSKQEVMSSFLPEGGCYELLTVIGKGFEDLMTVNLARYKP** **TGEYVTVRRINLEACSNEMVTFLQGELHVSKLFNHPNIVPYRATFIAD** **NELWVVTSFMAYGSAKDLICTHFMDGMNELAIAYILQGVLKALDYIHH** **MGYVHRSVKASHILISVDGKVYLSGLRSNLSMISHGQRQRVVHDFPKY** **SVKVLPWLSPEVLQQNLQGYDAKSDIYSVGITACELANGHVPFKDMPA** **TQMLLEKLNGTVPCLLDTSTIPAEELTMSPSRSVANSGLSDSLTTSTP** **RPSNGDSPSHPYHRTFSPHFHHFVEQCLQRNPDARPSASTLLNHSFFK** **QIKRRASEALPELLRPVTPITNFEGSQSQDHSGIFGLVTNLEELEVDD** **WEF**
Native sequence	Amino acids M1 – F431 (end) of human STRAD alpha. Residue M245 of fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	Prescission site (LEVLFQGP) at residues 221 – 228
Cloning sites	*Eco*R1 sites of pGex6P-1

Nucleotide sequence of insert

gaattcgcccttggatccATGTCATTTCTTGTAAGTAAACCAGAGCGA
ATCAGGCGGTGGGTCTCGGAAAAGTTCATTGTTGAGGGCTTAAGAGAT
TTGGAACTATTTGGAGAGCAGCCTCCGGGTGACACTCGGAGAAAAACC
AATGATGCGAGCTCAGAGTCAATAGCATCCTTCTCTAAACAGGAGGTC
ATGAGTAGCTTTCTGCCAGAGGGAGGGTGTTACGAGCTGCTCACTGTG
ATAGGCAAAGGATTTGAGGACCTGATGACTGTGAATCTAGCAAGGTAC
AAACCAACAGGAGAGTACGTGACTGTACGGAGGATTAACCTAGAAGCT
TGTTCCAATGAGATGGTAACATTCTTGCAGGGCGAGCTGCATGTCTCC
AAACTCTTCAACCATCCCAATATCGTGCCATATCGAGCCACTTTTATT
GCAGACAATGAGCTGTGGGTTGTCACATCATTCATGGCATACGGTTCT
GCAAAAGATCTCATCTGTACACACTTCATGGATGGCATGAATGAGCTG
GCGATTGCTTACATCCTGCAGGGGGTGCTGAAGGCCCTCGACTACATC
CACCACATGGGATATGTACACAGGAGTGTCAAAGCCAGCCACATCCTG
ATCTCTGTGGATGGGAAGGTCTACCTGTCTGGTTTGCGCAGCAACCTC
AGCATGATAAGCCATGGGCAGCGGCAGCGAGTGGTCCACGATTTTCCC
AAGTACAGTGTCAAGGTTCTGCCGTGGCTCAGCCCCGAGGTCCTCCAG
CAGAATCTCCAGGGTTATGATGCCAAGTCTGACATCTACAGTGTGGGA
ATCACAGCCTGTGAACTGGCCAACGGCCATGTCCCCTTTAAGGATATG
CCTGCCACCCAGATGCTGCTAGAGAAACTGAACGGCACAGTGCCCTGC
CTGTTGGATACCAGCACCATCCCCGCTGAGGAGCTGACCATGAGCCCT
TCGCGCTCAGTGGCCAACTCTGGCCTGAGTGACAGCCTGACCACCAGC
ACCCCCCGGCCCTCCAACGGTGACTCGCCCTCCCACCCCTACCACCGA
ACCTTCTCCCCCCACTTCCACCACTTTGTGGAGCAGTGCCTTCAGCGC
AACCCGGATGCCAGGCCCAGTGCCAGCACCCTCCTGAACCACTCTTTC
TTCAAGCAGATCAAGCGACGTGCCTCAGAGGCTTTGCCCGAATTGCTT
CGTCCTGTCACCCCCATCACCAATTTTGAGGGCAGCCAGTCTCAGGAC
CACAGTGGAATCTTTGGCCTGGTAACAAACCTGGAAGAGCTGGAGGTG
GACGATTGGGAGTTCtgaaagggcgaattc