Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of STRAD alpha [54 - 431] G76D G78D K197E

Enzyme description:- STRAD alpha [54 - 431] G76D G87D K197E

Clone number:- DU 17597

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST and His6

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 71, 130.73 daltons
Average Mass 71, 176.52 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.89

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

STRAD alpha [54 - 431] G76D G78D K197E

Protein	STRAD alpha [54 - 431] G76D G78D K197E
Clone number	DU 17597
Species	Human
Accession number	NM_001003787.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLVPRGSATMAHHHHHHALDLEVLFQ GPL**SKQEVMSSFLPEGGCYELLTVIDKDFEDLMTVNLARYKPTGEYVTV** **RRINLEACSNEMVTFLQGELHVSKLFNHPNIVPYRATFIADNELWVVTS** **FMAYGSAKDLICTHFMDGMNELAIAYILQGVLKALDYIHHMGYVHRSVE** **ASHILISVDGKVYLSGLRSNLSMISHGQRQRVVHDFPKYSVKVLPWLSP** **EVLQQNLQGYDAKSDIYSVGITACELANGHVPFKDMPATQMLLEKLNGT** **VPCLLDTSTIPAEELTMSPSRSVANSGLSDSLTTSTPRPSNGDSPSHPY** **HRTFSPHFHHFVEQCLQRNPDARPSASTLLNHSFFKQIKRRASEALPEL** **LRPVTPITNFEGSQSQDHSGIFGLVTNLEELEVDDWEF**
Native sequence	Amino acids S54 – F431 (end) of human STRAD alpha. Residue S249 of the fusion protein is equivalent to S54 of the native enzyme. The GST tag is located at residues 1 – 220 and the His6 tag is located at residues 231 - 236. The enzyme has G76D G78D and K197E mutations. Residue G76 is equivalent to D271 of the fusion protein. Residue G78 is equivalent to D273 of the fusion protein. Residue K197 is equivalent to E392 of the fusion protein.
Protease cleavage	PreScission (LEVLFQGP) residues 240 - 247
Cloning sites	*Bam*H1 and *EcoR*1 sites of pGEX4T-1

Nucleotide Sequence of Insert

ggatccgccaccatggcacatcatcatcaccatcacgcactggatc
tggaagttctgttccaggggcccctgTCTAAACAGGAGGTCATGAG
TAGCTTTCTGCCAGAGGGAGGGTGTTACGAGCTGCTCACTGTGATA
GACAAAGACTTTGAGGACTTGATGACTGTGAATCTAGCAAGGTACA
AACCAACAGGAGAGTACGTGACTGTACGGAGGATTAACCTAGAAGC
TTGTTCCAATGAGATGGTAACATTCTTGCAGGGCGAGCTGCATGTC
TCCAAACTCTTCAACCATCCCAATATCGTGCCATATCGAGCCACTT
TTATTGCAGACAATGAGCTGTGGGTTGTCACATCATTCATGGCATA
CGGTTCTGCAAAAGATCTCATCTGTACACACTTCATGGATGGCATG
AATGAGCTGGCGATTGCTTACATCCTGCAGGGGGTGCTGAAGGCCC
TCGACTACATCCACCACATGGGATATGTACACAGGAGTGTCGAAGC
CAGCCACATCCTGATCTCTGTGGATGGGAAGGTCTACCTGTCTGGT
TTGCGCAGCAACCTCAGCATGATAAGCCATGGGCAGCGGCAGCGAG
TGGTCCACGATTTTCCCAAGTACAGTGTCAAGGTTCTGCCGTGGCT
CAGCCCCGAGGTCCTCCAGCAGAATCTCCAGGGTTATGATGCCAAG
TCTGACATCTACAGTGTGGGAATCACAGCCTGTGAACTGGCCAACG
GCCATGTCCCCTTTAAGGATATGCCTGCCACCCAGATGCTGCTAGA
GAAACTGAACGGCACAGTGCCCTGCCTGTTGGATACCAGCACCATC
CCCGCTGAGGAGCTGACCATGAGCCCTTCGCGCTCAGTGGCCAACT
CTGGCCTGAGTGACAGCCTGACCACCAGCACCCCCCGGCCCTCCAA
CGGTGACTCGCCCTCCCACCCCTACCACCGAACCTTCTCCCCCCAC
TTCCACCACTTTGTGGAGCAGTGCCTTCAGCGCAACCCGGATGCCA
GGCCCAGTGCCAGCACCCTCCTGAACCACTCTTTCTTCAAGCAGAT
CAAGCGACGTGCCTCAGAGGCTTTGCCCGAATTGCTTCGTCCTGTC
ACCCCCATCACCAATTTTGAGGGCAGCCAGTCTCAGGACCACAGTG
GAATCTTTGGCCTGGTAACAAACCTGGAAGAGCTGGAGGTGGACGA
TTGGGAGTTCtgaaagggcgaattc

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