

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of STRAD alpha [54 - 431]

Enzyme description:- STRAD alpha [54 - 431]

Clone number:- DU 2148

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST and His6

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 71, 013.77 daltons

Average Mass 71, 059.50 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.08

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]

<u>Protein</u>	STRAD alpha [54 - 431]
<u>Clone number</u>	DU 2148
<u>Species</u>	Human
<u>Accession number</u>	NM_001003787.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPHYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPKSDLVPRGSATMAHHHHHHALDLEVLFO GPLSKQEVMS SFLPEGGCYELLTVIGKGFEDLMTVNLARYKPTGEYVTV RRINLEACSNEMVTFLOGELHVSKLFNHPNIVPYRATFIADNELWVVTS FMAYGSAKDLICTHFMDGMNELAIAYILQGVLKALDYIHHMGYVHRSVK ASHILISVDGKVYLSGLRSNLSMISHGQRQRVVHDFPKYSVKVLPWLS P EVLQONLQGYDAKSDIYSVGITACELANGHVVPFKDMPATQMLLEKLNGT VPCLLDTSTIPAEELTMSPSRSVANSGLSDSLTTSTPRPSNGDSPSHPY HRTFSPHFHFVEQCLQRNP DARPSASTLLNHSFFKQIKRRASEALPEL LRPVTPIITNFEQS QDHS GIFGLVTNLEEEVDDWEF</p>
<u>Native sequence</u>	<p>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.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFOGP</u>) residues 240 - 247
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Eco</i> R1 sites of pGEX4T-1

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Nucleotide
Sequence
of Insert

ggatccgccaccatggcacatcatcatcaccatcacgcactggatc
tggagttctgttccaggggccctgTCTAAACAGGAGGTCATGAG
TAGCTTTCTGCCAGAGGGAGGGTGTACGAGCTGCTCACTGTGATA
GGCAAAGGATTTGAGGACCTGATGACTGTGAATCTAGCAAGGTACA
AACCAACAGGAGAGTACGTGACTGTACGGAGGATTAACCTAGAAGC
TTGTTCCAATGAGATGGTAACATTTCTTGCAGGGCGAGCTGCATGTC
TCCAAACTCTTCAACCATCCCAATATCGTGCCATATCGAGCCACTT
TTATTGCAGACAATGAGCTGTGGGTTGTACATCATTTCATGGCATA
CGGTTCTGCAAAGATCTCATCTGTACACACTTCATGGATGGCATG
AATGAGCTGGCGATTGCTTACATCCTGCAGGGGGTGTGAAGGCC
TCGACTACATCCACCACATGGGATATGTACACAGGAGTGTCAAAGC
CAGCCACATCCTGATCTCTGTGGATGGGAAGGTCTACCTGTCTGGT
TTGCGCAGCAACCTCAGCATGATAAGCCATGGGCAGCGCAGCGAG
TGGTCCACGATTTTCCCAAGTACAGTGTCAAGGTTCTGCCGTGGCT
CAGCCCCGAGGTCTCCAGCAGAATCTCCAGGGTTATGATGCCAAG
TCTGACATCTACAGTGTGGGAATCACAGCCTGTGAACTGGCCAACG
GCCATGTCCCCTTTAAGGATATGCCTGCCACCCAGATGCTGCTAGA
GAAACTGAACGGCACAGTGCCCTGCCTGTTGGATAACCAGCACCATC
CCCGCTGAGGAGCTGACCATGAGCCCTTCGCGCTCAGTGGCCAAC
CTGGCCTGAGTGACAGCCTGACCACCAGCACCCCCGGCCCTCCAA
CGGTGACTCGCCCTCCCACCCCTACCACCGAACCTTCTCCCCCAC
TTCCACCACCTTGTGGAGCAGTGCCTTCAGCGCAACCCGGATGCCA
GGCCCAGTGCCAGCACCTCCTGAACCACTCTTTCTTCAAGCAGAT
CAAGCGACGTGCCTCAGAGGCTTTGCCCGAATTGCTTCGTCTCTGTC
ACCCCATCACCAATTTTGGAGGCGAGCCAGTCTCAGGACCACAGTG
GAATCTTTGGCCTGGTAACAAACCTGGAAGAGCTGGAGGTGGACGA
TTGGGAGTTctgaagggcgaattc

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