

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active LKB1 complex LKB1 [1 - 433] / MO25 [1 – 341] /STRAD [1 – 431]

<u>Enzyme description:-</u>	LKB1[1 - 433] MO25 alpha [1 – 341] STRAD alpha [1 – 431]
<u>Clone number:-</u>	LKB1 / STRAD / MO25 [DU 13002]
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	LKB1 N-terminal His(6) MO25 alpha Untagged STRAD alpha Untagged
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	3 mg/L
<u>Calculated molecular mass:-</u>	[cysteines reduced, methionines have not been oxidised]
Monoisotopic Mass: 50, 480.51 daltons [LKB1], 48, 338.30 daltons [STRAD], 39, 843.74 daltons [MO25]	
Average Mass: 50, 512.85 daltons [LKB1], 48, 369.00 daltons [STRAD], 39, 868.99 dalons [MO25]	
<u>Theoretical pI:-</u>	LKB1 = 6.97, STRAD = 6.02 MO25 = 6.43
<u>Purity:-</u>	>80 %
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.2 mM PMSF, 1 mM Benzamidine.	
<u>Storage temperature:-</u>	-70 °C [Long term stability to be determined]
<u>Assay:-</u>	Standard filter binding assay

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Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-

LSNLYHQGKFLQTFCGSPLYRRR

Final concentration: 300 μ M

[Residues 196 – 215 of human NUA2, plus 3 additional arginine residues]

Specific activity range:-

To be determined

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Clone Data Sheet

LKB1 [1 - 433]

<u>Protein</u>	LKB1 [1 - 433]
<u>Clone number</u>	DU 13002
<u>Species</u>	Human
<u>Accession number</u>	NP_000446
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MAHHHHHHENLYFQGMEVVDPPQQLGMFTEGELMSVGMDTFIHRIDS TEVIYQPRRKRAKLIGKYLMGDLLGEGSYGKVKEVLDSETLCRRAV KILKKKKLRRIPNGEANVKKEIQLLRRLRHKNVIQLVDVLYNEEKQ KMYMMEYCVCGMQEMLDSVPEKRFPVCQAHGYFCQLIDGLEYLHS QGIVHKDIKPGNLLLTGGLTKISDLGVAEALHPFAADDTCRTSQG SPAFQOPPEIANGLDTFSGFKVDIWSAGVTLYNITTGLYPFEGDNIY KLFENIGKGSYAIPGDCGPPLSDLLKGMLEYEPAKRFSIRQIRQHS WFRKKHPPAEAPVPIPPSPDTKDRWRSMTVVPYLEDLHGADEDEDL FDIEDDIIYTQDFTVPGQVPPEEEASHNGQRRGLPKAVCMNGTEAAQ LSTKSRAEGRAPNPARKACSASSKIRRLSACKQQ
<u>Native sequence</u>	Amino acids M1 – Q433 (end) of human LKB1. Residue M16 of the fusion protein is equivalent to M1 of the native enzyme. The fusion protein contains an N-terminal His(6) tag which is located at residues 3 - 8.
<u>Protease cleavage</u>	rTEV (ENLYFQG) residues 9 - 15

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Complete nucleotide sequence

cggaattctataaatATGGCACACCATCACCATCACCATGAAAACCTGT
ATTTTCAGGGCATGGAGGTGGTGGACCCGCAGCAGCTGGGCATGTTAC
GGAGGGCGAGCTGATGTCGGTGGGTATGGACACGTTTCATCCACCGCATC
GACTCCACCGAGGTCATCTACCAGCCGCGCCGCAAGCGGGCCAAGCTCA
TCGGCAAGTACCTGATGGGGGACCTGCTGGGGGAAGGCTCTTACGGCAA
GGTGAAGGAGGTGCTGGACTCGGAGACGCTGTGCAGGAGGGCCGTCAAG
ATCCTCAAGAAGAAGAAGTTGCGAAGGATCCCCAACGGGGAGGCCAACG
TGAAGAAGGAAATTCAACTACTGAGGAGGTTACGGCACAAAAATGTCAT
CCAGCTGGTGGATGTGTTATACAACGAAGAGAAGCAGAAAATGTATATG
GTGATGGAGTACTGCGTGTGTGGCATGCAGGAAATGCTGGACAGCGTGC
CGGAGAAGCGTTTCCAGTGTGCCAGGCCACGGGTACTTCTGTCAGCT
GATTGACGGCCTGGAGTACCTGCATAGCCAGGGCATTGTGCACAAGGAC
ATCAAGCCGGGGAACCTGCTGCTCACCACCGGTGGCACCCCTCAAATCT
CCGACCTGGGCGTGGCCGAGGCACTGCACCCGTTTCGCGGCGGACGACAC
CTGCCGGACCAGCCAGGGCTCCCCGGCTTTCCAGCCGCCCGAGATTGCC
AACGGCCTGGACACCTTCTCCGGCTTCAAGGTGGACATCTGGTCGGCTG
GGGTCACCCCTCTACAACATCACCACGGGTCTGTACCCCTTCGAAGGGGA
CAACATCTACAAGTTGTTTGAGAACATCGGGAAGGGGAGCTACGCCATC
CCGGGCGACTGTGGCCCCCGCTCTCTGACCTGCTGAAAGGGATGCTTG
AGTACGAACCGGCCAAGAGGTTCTCCATCCGGCAGATCCGGCAGCACAG
CTGGTTCCGGAAGAAACATCCTCCGGCTGAAGCACCAGTGCCCATCCCA
CCGAGCCCAGACACCAAGGACCGGTGGCGCAGCATGACTGTGGTGCCGT
ACTTGGAGGACCTGCACGGCGCGGACGAGGACGAGGACCTCTTCGACAT
CGAGGATGACATCATCTACACTCAGGACTTCACGGTGCCCGGACAGGTC
CCAGAAGAGGAGGCCAGTCACAATGGACAGCGCCGGGGCCTCCCCAAGG
CCGTGTGTATGAACGGCACAGAGGCGGCGCAGCTGAGCACCAAATCCAG
GGCGGAGGGCCGGGCCCCCAACCCTGCCCAGCAAGGCCTGCTCCGCCAGC
AGCAAGATCCGCCGGCTGTTCGGCTGCAAGCAGCAGtgaaagggcgaat
tc

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Clone Data Sheet

MO25 alpha [1 - 341]

<u>Protein</u>	MO25 alpha [1 – 341]
<u>Clone number</u>	DU 13002
<u>Species</u>	Human
<u>Accession number</u>	NP_057373
<u>Tags</u>	None
<u>Baculovirus expressed protein</u>	MPFPFGKSHKSPADIVKNLKESMAVLEKQDISDKKAEKATEEVSKNLVA MKEILYGTNEKEPQTEAVAQLAQELYNSGLLSTLVADLQLIDFEGKKDV AQIFNNILRRQIGTRTPTVEYICTQONILFMLLKGYESPEIALNCGIML RECIRHEPLAKIILWSEQFYDFFRYVEMSTFDIASDAFATFKDLLTRHK LLSAEFLEQHYDRFFSEYEKLLHSENYVTKRQSLKLLGELLLDRHNFTI MTKYISKPENLKLMMNLLRDKSRNIQFEAFHVFKVVFVANPNKTQPILDI LLKNQAKLIEFLSKFQNDRTEDeqFNDEKTYLVKQIRDLKRPAQqEA
<u>Native sequence</u>	Amino acids M1 – A341 (end) of human M025 alpha.
<u>Protease cleavage</u>	None

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Complete nucleotide sequence

tcgagtataaatATGCCGTTCCCGTTTGGGAAGTCTCACAAATCTCCAG
CAGACATTGTGAAGAATCTGAAGGAGAGCATGGCTGTTCTGGAAAAGCA
AGACATTTCTGATAAAAAAGCAGAAAAGGCTACAGAAGAAGTTTCCAAA
AATCTGGTTGCCATGAAAGAAATTCTGTATGGCACAAATGAAAAAGAGC
CTCAGACAGAAGCAGTAGCTCAACTTGCTCAAGAACTCTATAATAGTGG
GCTCCTTAGCACCCCTGGTAGCTGATTTACAGCTCATTGACTTTGAGGGC
AAAAAAGACGTGGCTCAAATTTTCAACAATATTCTCAGAAGACAAATTG
GTACGAGAACTCCTACTGTTGAATACATCTGCACCCAACAGAATATTTT
GTTTCATGTTATTGAAAGGGTATGAATCTCCAGAAATAGCTCTAAATTGT
GGAATAATGTTAAGAGAATGCATCAGACATGAACCACTTGCAAAAATCA
TTTTGTGGTCGGAACAGTTTTATGATTTCTTCAGATATGTCGAAATGTC
AACATTTGACATAGCTTCAGATGCATTTGCCACATTCAAGGATTTACTT
ACAAGACATAAATTGCTCAGTGCAGAATTTTTTGGAACAGCATTATGATA
GATTTTTTCAGTGAATATGAGAAGTTACTTCATTCAGAAAATTATGTGAC
AAAAAGACAGTCACTGAAGCTTCTCGGTGAACTACTACTAGATAGACAC
AACTTCACAATTATGACAAAATACATCAGTAAACCTGAGAACCTCAAAT
TAATGATGAACCTGCTGCGAGACAAAAGTCGCAACATCCAGTTTGAGGC
CTTTCACGTTTTTTAAGGTGTTTGTAGCCAATCCTAACAAGACGCAGCCC
ATCCTAGACATCCTCCTCAAGAACCAGGCCAACTCATAGAGTTCCTCA
GCAAGTTTCAGAACGACAGGACGGAGGATGAGCAGTTTAACGACGAGAA
GACCTATTTAGTTAAACAGATCAGGGATTTGAAGAGACCAGCTCAGCAA
GAAGCTtaagcatgcc

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Clone Data Sheet

STRAD alpha [1 - 431]

Protein STRAD alpha [1 – 431]

Clone number DU 13002

Species Human

Accession number AF308302

Tags None

**Baculovirus
expressed
protein**

**MSFLVSKPERIRRWVSEKFIVEGLRDLELFGEQPPGDTRRKTNDASSES
IASFSKQEVMSFLPEGGCYELLTVIGKGFEDLMTVNLARYKPTGEYVT
VRRINLEACSNEVMVTFLOGELHVSCLFNHPNIVPYRATFIADNELWVVT
SFMAYGSAKDLICTHFMDSMNEIAIAYILQGVKALDYIHHMGYVHRVS
KASHILISVDGKVYLSGLRSNLSMISHGQRQRVVHDFPKYSVKVLPWLS
PEVLQONLQGYDAKSDIYSGITACELANGHVFPKDMPATQMLLEKLN
TVPCLLDTSTIPAEELTMSPSRSVANGLSDSLTTSTPRPSNGDSPSHP
YHRTFSPHFHHFVEQCLQRNPDARPSASTLLNHSFFKQIKRRASEALPE
LLRPVTPITNFEQSQSDHSGIFGLVTNLEELEVDWEF**

Native sequence Amino acids M1 – F431 (end) of human STRAD alpha.

The following amino acid substitutions are present:

W – S, where W339 of the native protein is S577 of the fusion protein

K – E, where K388 of the native protein is E626 of the fusion protein

Protease cleavage None

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Complete nucleotide sequence

aattctataaatATGTCATTTCTTGTAAGTAAACCAGAGCGAATCAGGC
GGTGGGTCTCGGAAAAGTTCATTGTTGAGGGCTTAAGAGATTTGGAAC
ATTTGGAGAGCAGCCTCCGGGTGACACTCGGAGAAAAACCAATGATGCG
AGCTCAGAGTCAATAGCATCCTTCTCTAAACAGGAGGTCATGAGTAGCT
TTCTGCCAGAGGGAGGGTGTTACGAGCTGCTCACTGTGATAGGCAAAGG
ATTTGAGGACCTGATGACTGTGAATCTAGCAAGGTACAAACCAACAGGA
GAGTACGTGACTGTACGGAGGATTAACCTAGAAGCTTGTTCCAATGAGA
TGGTAACATTCTTGCAGGGCGAGCTGCATGTCTCCAAACTCTTCAACCA
TCCCAATATCGTGCCATATCGAGCCACTTTTATTGCAGACAATGAGCTG
TGGGTTGTCAATCATTCATGGCATAACGGTTCTGCAAAAGATCTCATCT
GTACACACTTCATGGATGGCATGAATGAGCTGGCGATTGCTTACATCCT
GCAGGGGGTGCTGAAGGCCCTCGACTACATCCACCACATGGGATATGTA
CACAGGAGTGTCAAAGCCAGCCACATCCTGATCTCTGTGGATGGGAAGG
TCTACCTGTCTGGTTTGCAGCAACCTCAGCATGATAAGCCATGGGCA
GCGGCAGCGAGTGGTCCACGATTTTCCCAAGTACAGTGTCAAGGTTCTG
CCGTGGCTCAGCCCCGAGGTCCTCCAGCAGAATCTCCAGGGTTATGATG
CCAAGTCTGACATCTACAGTGTGGGAATCACAGCCTGTGAACTGGCCAA
CGGCCATGTCCCCTTTAAGGATATGCCTGCCACCCAGATGCTGCTAGAG
AAACTGAACGGCACAGTGCCCTGCCTGTTGGATAACCAGCACCATCCCCG
CTGAGGAGCTGACCATGAGCCCTTCGCGCTCAGTGGCCAACCTCTGGCCT
GAGTGACAGCCTGACCACCAGCACCCCCCGGCCCTCCAACGGTGACTCG
CCCTCCCACCCCTACCACCGAACCTTCTCCCCCACTTCCACCCTTTG
TGGAGCAGTGCCTTCAGCGCAACCCGGATGCCAGGCCAGTGCCAGCAC
CCTCCTGAACCACTCTTTCTTCAAGCAGATCAAGCGACGTGCCTCAGAG
GCTTTGCCCGAATTGCTTCGTCCTGTCACCCCCATCACCAATTTTGAGG
GCAGCCAGTCTCAGGACCACAGTGAATCTTTGGCCTGTAACAAACCT
GGAAGAGCTGGAGGTGGACGATTGGGAGTTCTgagcgccgc