

Certificate of Analysis

LKB1/STRAD α /MO25 α , active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-596, 14-596-K, 14-596M

Parent Lot # 1689134

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: Complex of *N*-terminal 6His-tagged, recombinant, full length, human LKB1, *N*-terminal GST-tagged, recombinant, human, full length STRAD α and *N*-terminal GST-tagged, recombinant, human, full length MO25 α , expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose followed by Ni²⁺/NTA agarose. Purity 99% by SDS-PAGE and Coomassie blue staining. LKB1 MW = 52.1kDa, STRAD α MW = 76.1kDa, MO25 α MW = 66.8kDa.

Specific Activity (Parent lot# 1689134): 22U/mg, where one unit of LKB1/STRAD α /MO25 α , active activity is defined as 1nmol phosphate incorporated into 300 μ M (LSNLYHQGKFLQTFCGSPLYRRR) per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.806mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

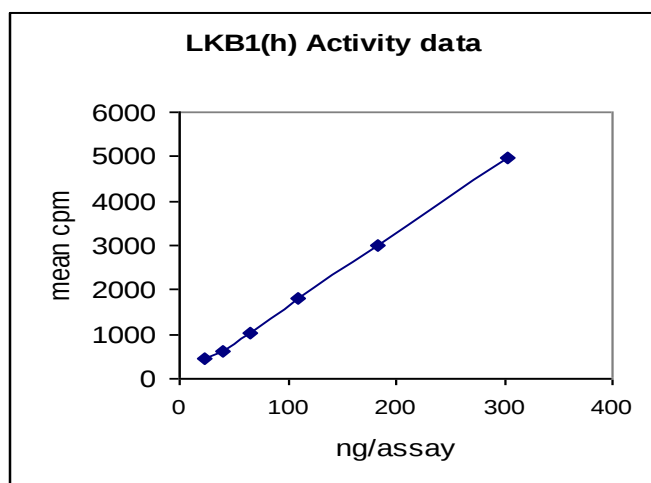
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

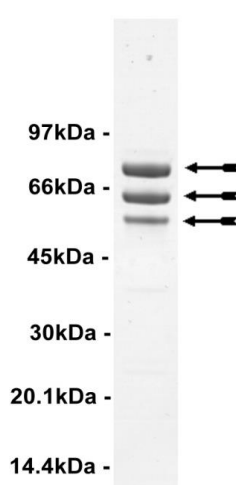
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 24–303ng of this lot of enzyme phosphorylated 300 μ M (LSNLYHQGKFLQTFCGSPLY) RRR in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as LKB1 with the translated native sequence listed on page three, STRAD α with the translated native sequence listed on page four and MO25 α with the translated native sequence listed on page six.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of LKB1/STRAD α /MO25 α , active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(LSNLYHQGKFLQTFGSPLYRRR):** Use at a final assay concentration of 300µM. Make up a 3mM stock. Add 2.5µl of stock per assay point.
3. **Triton X-100:** Make up a 1% (w/v) stock. Add 2.5µl of stock per assay point.
4. **LKB1/STRADα/MO25α, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 24–303ng per assay point.
5. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure:

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(LSNLYHQGKFLQTFGSPLYRRR)**.
3. Add 2.5µl of 1% (w/v) Triton X-100.
4. Add **2.5µl (24–303ng) LKB1/STRADα/MO25α active**.
5. Add 2.5µl of dH₂O.
6. Add 10µl of diluted [γ-³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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LKB1 Sequence Information

Protein	Human LKB1
Tags	N-terminal 6His
Native sequence	M29 of the recombinant protein is equivalent to M1 of human LKB1
Accession number	GenBank NM000455

Recombinant LKB1 amino acid sequence:

```

1 MSYYHHHHHH DYDIPTTENL YFQGAMGSME VDPQQLGMF TEGELMSVGM DTFIHRIDST
61 EVIYQPRRKR AKLIGKYLGM DLLGEGSYGK VKEVLDSETL CRRAVKILKK KKLRRIPNGE
121 ANVKKEIQLL RRLRHKNVIQ LVDVLYNEEK QKMYMVMMEYC VCGMQEMLDS VPEKRFVPCQ
181 AHGYFCQLID GLEYLHSQGI VHKDIKPGNL LLTTGGTLKI SDLGVAEALH PFAADDTCRT
241 SQGSPAFQPP EIANGLDTFS GFKVDIWSAG VTLYNITTGL YPFEGDNIYK LFENIGKGSY
301 AIPGDCGPPL SDLLKGMLEY EPAKRFSIRQ IRQHSWFRKK HPPAEAPVPI PPSPDTKDRW
361 RSMTVVPYLE DLHGAEDED LFDIEDDIY TQDFTVPGQV PEEEAASHNGQ RRGLPKAVCM
421 NGTEAAQLST KSRAEGRAPN PARKACSASS KIRRLSACKQ Q

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Recombinant LKB1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacgaat ggctgatagt
121 ggcttagata aaaaatccac aaaatgcccc gactgttcat ctgcttctca gaaagatgta
181 ctttgtgtat gttccagcaa aacaagggtt cctccagttt tgggtggtgga aatgtcacag
241 acatcaagca ttggtagtgc agaactctta atttacttgg agagaaaaaa agaaaaaat
301 atcaacagag atataacctc caggaagatg ttgccctcaa gaacctcaa tgtagagaga
361 aaagcatctc agcaacaatg gggtcggggc aactttacag aaggaagaat tcctcacata
421 aggattgaga atggagctgc tattgaggaa atctatacct ttggaagaat attgggaaaa
481 gggagctttg gaatagtcac tgaagcgaca gacaaggaaa cagaaacgaa gtgggcaatt
541 aaaaaagtga acaaaagaaa ggctggaagc tctgctgtga agttacttga acgagagggtg
601 aacattctga aaagtgtaaa acatgaacac atcatacatc tggaacaagt atttgaaacg
661 ccaaagaaaa tgtaccttgt gatggagctt tgtgaggatg gagaactcaa agaaattctg
721 gataggaaag ggcatttctc agagaatgag acaagggtga tcattcaaag tctcgcatac
781 gctatagcat atcttcacaa taatgatatt gtacatagag atctgaaact ggaaaatata
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901 gatgttggct tagcggtgaa gaagcaaagt aggagtgaag ccatgctgca ggccacatgt
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1201 gatcctgctc acagaatcac agctaaggaa ctactagata accagtgggt aacaggcaat
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1321 ccagaaagtg ttgaggaaaa cacaacagaa gagaagaata agccgtccac tgaagaaaag
1381 ttgaaaagtt accaaccctg gggaatgtc cctgatgcca attacacttc agatgaagag
1441 gaggaaaaac agtctactgc ttatgaaaag caatttcctg caaccagtaa ggacaacttt
1501 gatatgtgca gttcaagttt cacatctagc aaactcctc cagctgaaat caaggagaga
1561 atggagaaaa cccctgtgac tccaagccaa ggaacagcaa ccaagtaccc tgctaaatcc
1621 ggcgccctgt ccagaaccaa aaagaaactc taa

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STRADα Sequence Information

Protein	Human STRADα
Tags	N-terminal GST
Native sequence	M239 of the recombinant protein is equivalent to M1 of human STRADα
Accession number	GenBank AF308302

Recombinant STRADα amino acid sequence:

```

1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSM  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSR  YSKDFETLKV
121  DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGPLG  SPEFALETMS
241  FLVSKPERIR  RWVSEKFIVE  GLRDLELFGE  QPPGDTRRKT  NDASSESIAS  FSKQEVMSSE
301  LPEGGCYELL  TVIGKGFEDL  MTVNLARYKP  TGEYVTVRRI  NLEACSNEMV  TFLQGELHVS
361  KLFNHPNIVP  YRATFIADNE  LWVVSFMY  GSAKDLICTH  FMDGMNELAI  AYILQGV LKA
421  LDYIHHMGYV  HRSVKASHIL  ISVDGKVYLS  GLRSNLSMIS  HGQRQRVVHD  FPKYSVKVLP
481  WLSPEVLQQN  LQGYDAKSDI  YSVGITACEL  ANGHVPFKDM  PATQMLLEKL  NGTVPCLLDT
541  STIPAEELTM  SPSRSVANS  LSDSLTSTP  RPSNGDSPSH  PYHRTFSPHF  HHFVEQCLQR
601  NPDARPSAST  LLNHSFFKQI  KRRASEALPE  LLRPVTPITN  FEQSQSDHS  GIFGLVTNLE
661  ELEVDWDF

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Recombinant STRADα nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatata  ttgaagaaa  atatgaagag  catttgatg  agcgcatga  aggtgataaa
121  tggcgaaaca  aaaagttga  attgggttg  gagtttcca  atcttcctta  ttatatgat
181  ggtgatgta  aattaacaca  gtctatggcc  atcatagct  atatactga  caagcacaac
241  atgttggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcgggtttg
301  gatattagat  acggtgttgc  gagaattgca  tatagtaaag  actttgaaac  tctcaaagtt
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421  acatatatta  atggtgatca  tgtaaccat  cctgacttca  tgttgtatga  cgctcttgat
481  gttgttttat  acatggacc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
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1141  ctgtgggttg  tcacatcatt  catggcatac  ggttctgcaa  aagatctcat  ctgtacacac
1201  ttcatggatg  gcatgaatga  gctggcgatt  gcttacatcc  tgcagggggg  gctgaaggcc
1261  ctgcactaca  tccaccacat  gggatatgta  cacaggagt  tcaaagccag  ccacatcctg
1321  atctctgtgg  atgggaagg  ctacgtgtct  ggtttgcgca  gcaacctcag  catgataagc
1381  catgggcagc  ggcagcgagt  ggtccacgat  tttcccaagt  acagtgtcaa  ggttctgccg
1441  tggctcagcc  ccgaggtcct  ccagcagaat  ctccagggtt  atgatgccaa  gtctgacatc
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1621  agcaccatcc  ccgctgagga  gctgaccatg  agcccttcgc  gctcagtggc  caactctggc
1681  ctgagtgaac  gcctgaccac  cagcaccctc  cggccctcca  acggtgactc  gccctccac
1741  ccctaccacc  gaaccttctc  cccctacttc  caccacttgc  tggagcagtg  ccttcagcgc
1801  aaccgggatg  ccaggcccag  tgcagcacc  ctctgaacc  actcttctt  caagcagatc

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1861 aagcgacgtg cctcagaggc ttgcccga ttgcttcgtc ctgtcacccc catcaccaat
1921 ttgaggggca gccagtctca ggaccacagt ggaatctttg gcctggtaac aaacctggaa
1981 gagctggagg tggacgattg ggagttctga
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MO25α Sequence Information

Protein	Human MO25α
Tags	N-terminal GST
Native sequence	M232 of the recombinant protein is equivalent to M1 of human MO25α
Accession number	GenBank NM_016289

Recombinant MO25α amino acid sequence:

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1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFGPLG SMPFPFGKSH
241 KSPADIVKNL KESMAVLEKQ DISDKKAKEA TEEVSKNLVA MKEILYGTNE KEPQTEAFAQ
301 LAQELYNSGL LSTLVADLQL IDFEGKKDVA QIFNNILRRQ IGTTRPTVEY ICTQQNILFM
361 LLKGYESPEI ALNCGIMLRE CIRHEPLAKI ILWSEQFYDF FRYVEMSTFD IASDAFATFK
421 DLLTRHKLLS AEFLEQHYDR FFSEYEKLLH SENYVTKRQS LKLLGELLDD RHNFTIMTKY
481 ISKPENLKLM MNLLRDKSRN IQFEAFHVFK VFVANPNKTQ PILDILLKNQ AKLIEFLSKF
541 QNDRTEDEQF NDEKTYLVKQ IRDLKRAQQ EA

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Recombinant MO25α nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61  ttggaatata ttgaagaaaa atatgaagag catttgatga agcgcatga aggtgataaa
121  tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatgtat
181  ggtgatgtta aattaacaca gtctatggcc atcatagctt atatagctga caagcacaac
241  atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301  gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361  gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
421  acatatatta atggtgatca tgtaaccat cctgacttca tgttgatga cgctcttgat
481  gttgttttat acatggagcc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541  aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601  tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
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721  aaatctccag cagacattgt gaagaatctg aaggagagca tggctgttct ggaaaagcaa
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901  ctgtctcaag aactctataa tagtgggctc cttagtacc tggtagctga tttacagctc
961  attgactttg agggcaaaaa agacgtggct caaatattca acaatattct cagaagacaa
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1621 cagaacgaca ggacggagga tgagcagttt aacgacgaga agacctattt agttaaacag
1681 atcagggatt tgaagagacc agctcagcaa gaagcttaa

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Reviewed and approved by site quality representative.

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