

Certificate of Analysis

LATS1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-988, 14-988-K, 14-988M

Parent Lot # D18EP019N

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: N-terminal GST-tagged, recombinant, human LATS1 amino acids 589-end and N-terminal 6His-tagged, recombinant, human MOBKL1A full length co-expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.

Complex activated *in vivo* with recombinant MST2. Purity 85% (combined) by SDS-PAGE and Coomassie blue staining. LATS1 MW = 90kDa, MOBKL1A MW = 30kDa.

Specific Activity (Parent lot# D18EP019N):

234U/mg, where one unit of LATS1 activity is defined as 1nmol phosphate incorporated into 250μM KKLNRTLSFAEPG per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.79mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

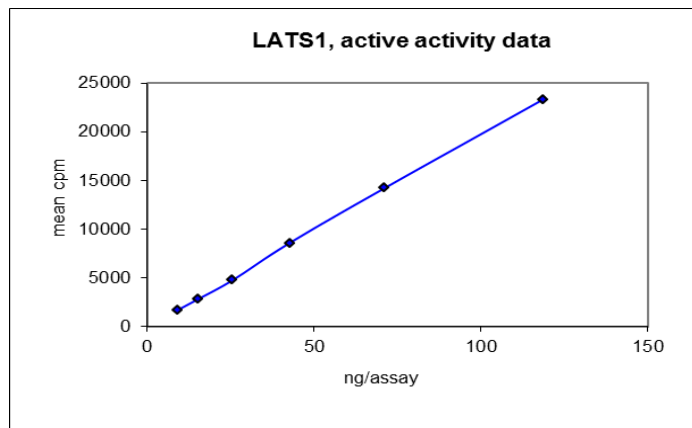
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

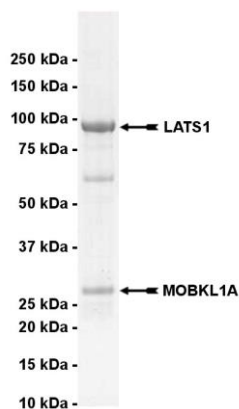
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 9.21–118.50ng of this lot of enzyme phosphorylated 250μM KKLNRTLSFAEPG 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 LATS1 and MOBKL1A with the translated sequences listed on pages three and five.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3μg of LATS1, active..

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. KKLNRTLSFAEPG:** Use at a final assay concentration of 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
- 3. LATS1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 9.21–118.50ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[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 (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of KKLNRTLSFAEPG.
3. Add **2.5 μ l (9.21–118.50ng) LATS1, active.**
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the dried filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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LATS1, active Sequence Information

<u>Protein</u>	Human LATS1
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	K231 of the recombinant protein is equivalent to K589 of human LATS1
<u>Accession number</u>	GenBank NM_004690.2

Recombinant LATS1 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 LEVLFQGPEF KEDESEKSYE
241 NVDSGDKEKK QITTSPITVR KNKKDEERRE SRIQSYSPQA FKFFMEQHVE NVLKSHQQL
301 HRKKQLENEM MRVGLSQDAQ DQMRKMLCQK ESNYIRLKRA KMDKSMFVKI KTLGIGAFGE
361 VCLARKVDTK ALYATKTLRK KDVLRLNQVA HVKAERDILA EADNEWVVRL YYSFQDKDNL
421 YFVMDYIPGG DMMSLLIRMG IFPESLARFY IAELTCAVES VHKMFGFIHRD IKPDNILIDR
481 DGHIKLTDGF LCTGFRWTHD SKYYQSGDHP RQDSMDFSNE WGD PSSCRG DRLKPLERRA
541 ARQHQRCLAH SLVGTPNYIA PEVLLRTGYT QLCDWWSVGV ILFEMLVGQP PFLAQTPLET
601 QMKVINWQTS LHIPPQAKLS PEASDLIIKL CRGPEDRLGK NGADEIKAHP FFKTIDFSSD
661 LRQQSASYIP KITHPTDTSN FDPVDPDKLW SDDNEEENVN DTLNGWYKNG KHPEHAFYEF
721 TFRRFDDNG YPYNYPKPIE YEYINSQGSE QQSEDDQNT GSEIKNRDLV YV

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Recombinant LATS1 nucleotide sequence:

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1  atgtccccta tactaggtta ttggaataat aagggccttg tgcaaccacac tcgacttctt
61  ttggaatata ttgaagaaaa atatgaagag catttgatag agcgcatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatgat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcgggtttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatataa atggtgatca tgtaacccat cctgacttca tggtgatga cgctcttgat
481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggaagtgc tgttccaggg gccgaattc aaggaagatg agagtgaata gagttatgaa
721 aatggtgata gtggggataa agaaaagaaa cagattacaa ctacacatat tactgttagg
781 aaaaacaaga aagatgaaga gcgaaggga tctcgtattc aaagttattc tcctcaagca
841 tttaaattct ttatggagca acatgtagaa aatgtactca aatctcatca gcagcgtcta
901 catcgtaaaa aacaattaga gaatgaaatg atgcggtttg gattatctca agatgccag
961 gatcaaatga gaaagatgct ttgccaaaaa gaatctaatt acatccgtct taaaagggct
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1141 aaagatgttc ttcttcgaaa tcaagtgcgt catgttaagg ctgagagaga tatcctggct
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1261 tactttgtaa tggactacat tcctgggggt gatattgatg gcctattaat tagaatgggc
1321 atctttccag aaagtctggc acgattctac atagcagaac ttacctgtgc agttgaaagt
1381 gttcataaaa tgggttttat tcatagagat attaaacctg ataataatctt gattgatcgt

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1441 gatggtcata ttaaattgac tgactttggc ctctgcactg gcttcagatg gacacacgat
1501 tctaagtact atcagagtgg tgaccatcca cggcaagata gcatggattt cagtaatgaa
1561 tggggggatc cctcaagctg tcgatgtgga gacagactga agccattaga gcggagagct
1621 gcacgccagc accagcgatg tctagcacat tctttgggtg ggactcccaa ttatattgca
1681 cctgaagtgt tgctacgaac aggatacaca cagttgtgtg attggtggag tgttgggtgt
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1801 caaatgaagg ttatcaactg gcaaacatct cttcacattc caccacaagc taaactcagt
1861 cctgaagctt ctgatcttat tattaaactt tgccgaggac ccgaagatcg cttaggcaag
1921 aatggtgctg atgaaataaa agctcatcca ttttttaaaa caattgattt ctccagtgc
1981 ctgagacagc agtctgcttc atacattcct aaaatcacac acccaacaga tacatcaaat
2041 tttgatcctg ttgatcctga taaattatgg agtgatgata acgaggaaga aaatgtaaat
2101 gacactctca atggatggta taaaaatgga aagcatcctg aacatgcatt ctatgaattt
2161 accttccgaa ggttttttga tgacaatggc taccatata attatccgaa gcctattgaa
2221 tatgaataca ttaattcaca aggctcagag cagcagtcgg atgaagatga tcaaacaca
2281 ggctcagaga ttaaaaatcg cgatctagta tatgttta

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MOBKL1A Sequence Information

<u>Protein</u>	Human MOBKL1A
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M37 of the recombinant protein is equivalent to M1 of human MOBKL1A
<u>Accession number</u>	GenBank BC038112.1

Recombinant MOBKL1A amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMSFL FGSRSSKTFK PKKNIPEGSH
61 QYELLKHAEA TLGSGNLRMA VMLPEGEDLN EWVAVNTVDF FNQINMLYGT ITDFCTEESC
121 PVMSAGPKYE YHWADGTNIK KPIKCSAPKY IDYLMTWVQD QLDDETLFPS KIGVPFPKNF
181 MSVAKTILKR LFRVYAHYH QHFDPVIQLQ EEHLNLSFK HFIFVQEFN LIDRRELAPL
241 QELIEKLTSK DR

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Recombinant MOBKL1A nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaaat gagcttcttg
121 tttggtagtc gctcttctaa aactttttaa ccaaagaaga acattccaga gggttctcac
181 cagtatgagc tcttaaaaca cgcagaagcc acacttggca gtggcaacct tcggatggct
241 gtcattgctt ctgaagggga agatctcaat gaatgggttg cagttaacac tgtggatttc
301 ttcaatcaga tcaacatgct ttatggaact atcacagact tctgtacaga agagagttgt
361 ccagtgatgt cagctggccc aaaatatgag tatcattggg cagatggaac gaacataaag
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481 cagttggatg atgagacgtt atttccatca aaaattggtg tcccgttccc aaagaatttc
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601 cagcattttg accctgtgat ccagcttcag gaggaagcac atctaaatac atctttcaag
661 cactttattt tttttgtcca ggaattcaac cttattgata gaagagaact tgcaccactc
721 caagaactga ttgaaaaact cacctcaaaa gacagataa

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