

Certificate of Analysis

LATS2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-987, 14-987-K, 14-987M

Parent Lot # D18EP020N

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 LATS2 amino acids 553-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 75% (combined) by SDS-PAGE and Coomassie blue staining. LATS2 MW = 89kDa, MOBKL1A MW = 30kDa.

Specific Activity (Parent lot# D18EP020N):

338U/mg, where one unit of LATS2 activity is defined as 1nmol phosphate incorporated into 250 μ M KKLNRTLSFAEPG per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.30mg/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.

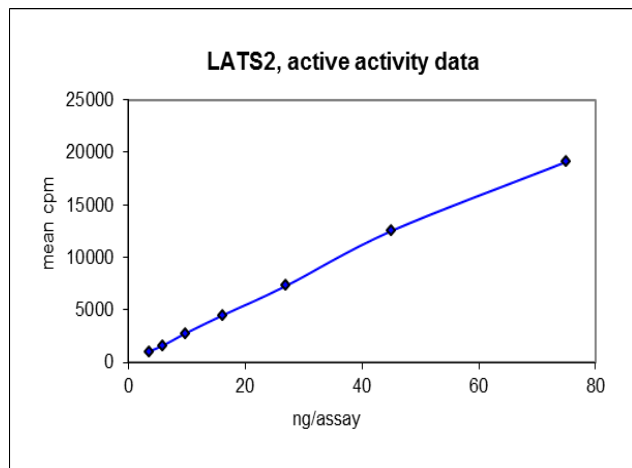
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

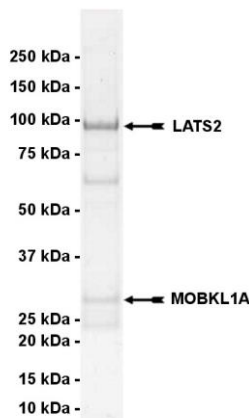
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 3.50–75.00ng of this lot of enzyme phosphorylated 250 μ M 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 LATS2 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 3 μ g of LATS2, 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. LATS2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 3.50-75.00ng 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 (3.50-75.00ng) LATS2, 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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LATS2, active Sequence Information

<u>Protein</u>	Human LATS2
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	G236 of the recombinant protein is equivalent to G553 of human LATS2
<u>Accession number</u>	GenBank NM_014572.2

Recombinant LATS2 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 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSKEFK GLRRQGGDKS
241 RKSAGDKGG KDKKQIQTSP VPVRKNSRDE EKRESRIKSY SPYAFKFFME QHVENVIKTY
301 QQKVNRRRLQL EQEMAKAGLC EAEQEQMRKI LYQKESNYNR LKRAKMDKSM FVKIKTLGIG
361 AFGEVCLACK VDTHALYAMK TLRKKDVLNR NQVAHVKAER DILAEADNEW VVKLYYSFQD
421 KDSLYFVM DY IPGGDMMSLL IRMEVFPEHL ARFYIAELTL AIESVHKMGF IHRDIKPDNI
481 LIDL DGH IKL TDFGLCTGFR WTHNSKYQK GSHVRQDSME PSDLWDDVSN CRCGDR LKTL
541 EQ RAR KQHQR CLAHSLVGTP NYIAPEVLLR KGYTQLCDWW SVGVILFEML VGQPPFLAPT
601 PTETQLKVIN WENTLHIPAQ VKLSPEAR DL ITKLCCSADH RLGRNGADDL KAHPPFFSAID
661 FSSDIRKQPA PYVPTISHPM DTSNFD PVDE ESPWNDASEG STKAWDTLTS PNNKHPEHAF
721 YEF TFR RFFD DNGYPFRCPK PSGAEASQAE SSDLESSDLV DQTEGCQPVY V

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Recombinant LATS2 nucleotide sequence:

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1  atgtccccta tactaggtta ttggaataat aagggccttg tgcaaccacac tcgacttctt
61  ttggaatata ttgaagaaaa atatgaagag catttgatg agcgcatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggttg gagtttcca 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 tgttgatga cgctcttgat
481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggttccgc gtggatccaa ggaattcaaa ggcctacgtc gacaaggcgg cgacaagagc
721 cgcaaaagcg ccaaggggga caaaggcgga aaggataaaa agcagattca gacctctccc
781 gttcccgtcc gcaaaaacag cagagacgaa gagaagagag agtcacgcat caagagctac
841 tcgccatacg cctttaagtt cttcatggag cagcacgtgg agaattgcat caaaacctac
901 cagcagaagg ttaaccggag gctgcagctg gagcaagaaa tggccaaagc tggactctgt
961 gaagctgagc aggagcagat gcggaagatc ctctaccaga aagagtctaa ttacaacagg
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1261 aaagacagcc tgtactttgt gatggactac atccctggtg gggacatgat gagcctgctg
1321 atccggatgg aggtcttccc tgagcacctg gcccggttct acatcgagca gctgactttg
1381 gccattgaga gtgtccacaa gatgggcttc atccaccgag acatcaagcc tgataacatt

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1441	ttgatagatc	tggatgggtca	cattaaactc	acagatttcg	gcctctgcac	tgggttcagg
1501	tggactcaca	attccaaata	ttaccagaaa	gggagccatg	tcagacagga	cagcatggag
1561	cccagcgacc	tctgggatga	tgtgtctaac	tgtcgggtgtg	gggacaggct	gaagacccta
1621	gagcagaggg	cgcggaagca	gcaccagagg	tgcttggcac	attcactggt	ggggactcca
1681	aactacatcg	cacccgaggt	gctcctccgc	aaaggggtaca	ctcaactctg	tgactgggtgg
1741	agtgttggag	tgattctctt	cgagatgctg	gtggggcagc	cgcccttttt	ggcacctact
1801	cccacagaaa	cccagctgaa	ggtgatcaac	tgggagaaca	cgctccacat	tccagcccag
1861	gtgaagctga	gccctgaggc	cagggacctc	atcaccaagc	tgtgctgctc	cgcagaccac
1921	cgcttggggc	ggaatggggc	cgatgacctg	aaggcccacc	ccttcttcag	cgccattgac
1981	ttctccagtg	acatccggaa	gcagccagcc	ccctacgttc	ccaccatcag	ccaccccatg
2041	gacacctcga	atttcgacct	cgtagatgaa	gaaagccctt	ggaacgatgc	cagcgaaggt
2101	agcaccaagg	cctgggacac	actcacctcg	ccaataaca	agcatcctga	gcacgcattt
2161	tacgaattca	ccttccgaag	gttctttgat	gacaatggct	acccctttcg	atgccccaaag
2221	ccttcaggag	cagaagcttc	acaggctgag	agctcagatt	tagaaagctc	tgatctggtg
2281	gatcagactg	aaggctgcca	gcctgtgtac	gtgtaa		

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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
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601 cagcattttg accctgtgat ccagcttcag gaggaagcac atctaaatac atctttcaag
661 cactttatth tttttgtcca ggaattcaac cttattgata gaagagaact tgcaccactc
721 caagaactga ttgaaaaact cacctcaaaa gacagataa

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