

Certificate of Analysis

LIM Kinase 1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-656, 14-656-K, 14-656M

Parent Lot # WAB0453

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 6His-tagged, recombinant, human LIM Kinase 1, amino acids 285–638, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺-NTA agarose. Activated using ROCKII (cat# 14-451) and repurified using Q Sepharose. Purity 70% by SDS-PAGE and Coomassie blue staining. MW = 41.2kDa.

Specific Activity (Parent lot# WAB0453): 1813U/mg, where one unit of LIM Kinase 1 activity is defined as 1nmol phosphate incorporated into 0.634mg/ml cofilin 1 per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.224mg/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.

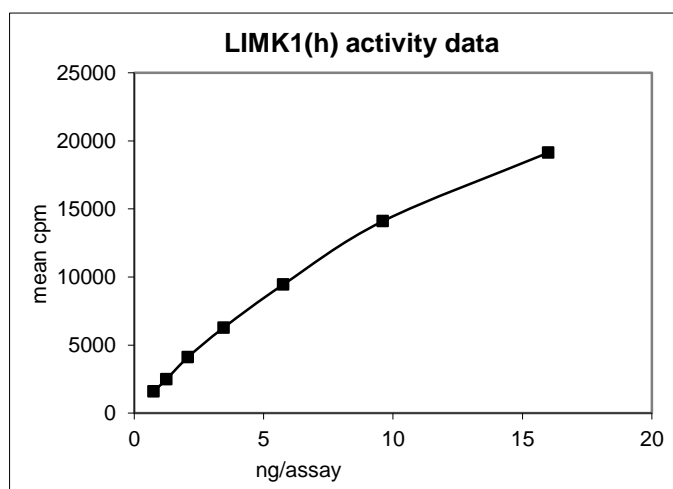
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

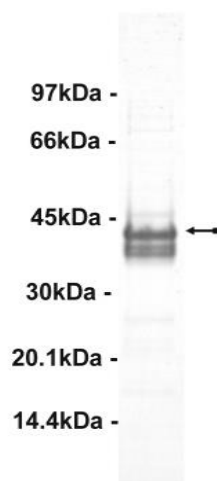
Quality Control Testing

Kinase Assay: 0.75–16ng of this lot of enzyme phosphorylated 0.634mg/ml Cofilin 1 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 product identity as LIM Kinase 1 with the translated native sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of LIM Kinase 1, active.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **Cofilin 1:** Use at a final concentration of 0.634mg/ml. Make a 3.17mg/ml stock. Add 5µl of stock per assay point.
3. **LIM Kinase 1, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.75–16ng per assay point.
4. **[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 (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 5µl of 3.17mg/ml **cofilin 1**.
3. Add **2.5µl (0.75–16ng) LIM Kinase 1, active**.
4. Add 2.5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 15 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl 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 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 1µl of 30% phosphoric acid.

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LIM Kinase 1 Sequence Information

Protein	Human LIM Kinase 1
Tags	N-terminal 6His
Native sequence	G10 of the recombinant protein is equivalent to G285 of human LIM Kinase 1
Accession number	GenBank NM_002314

Recombinant LIM Kinase 1 amino acid sequence:

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1  MHHHHHHEFG  SSARQKPVLR  SCSIDRSPGA  GSLGSPASQR  KDLGRSESLR  VVCRPHRIFR
61  PSDLIHGEVL  GKGCFGQAIK  VTHRETGEVM  VMKELIRFDE  ETQRTFLKEV  KVMRCLEHPN
121 VLKFIGVLYK  DKRLNFITEY  IKGGTLRGII  KSMDSQYPWS  QRVSFADKIA  SGMAYLHSMN
181 IIHRDLNSHN  CLVRENKNVV  VADFLARLM  VDEKTQPEGL  RSLKKPDRKK  RYTVVGNPYW
241 MAPEMINGRS  YDEKVDVFSF  GIVLCEIIGR  VNADPDYLPR  TMDFGLNVRG  FLDRYCPPNC
301 PPSFFPITVR  CCDLDPEKRP  SFVKLEHWLE  TLRMHLAGHL  PLGPQLEQLD  RGFWETYRRG
361  ESG

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Recombinant LIM Kinase 1 nucleotide sequence:

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1  atgcatcatc  accatcacca  tgaattcggc  agctctgccc  ggagaaaacc  tgtcttgagg
61  agctgcagca  tcgacaggct  tccgggcgct  ggctcactgg  gctccccggc  ctcccagcgc
121 aaggacctgg  gtcgctctga  gtccctccgc  gtagtctgcc  ggccacaccg  catcttcggg
181 ccgtcggacc  tcattccacg  ggaggtgctg  ggcaagggct  gcttcggcca  ggctatcaag
241 gtgacacacc  gtgagacagg  tgaggtgatg  gtgatgaagg  agctgatccg  gttcgacgag
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421 atcaagggcg  gcacgctccg  gggcatcatc  aagagcatgg  acagccagta  cccatggagc
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1081 gagagcggat  ga

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