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Certificate of Analysis

LIM Kinase 1, unactive

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-659, 14-659-K, 14-659M
Parent Lot # WAB0496

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 Ni^{2+}/NTA agarose. Purity 75% by SDS-PAGE and Coomassie blue staining. MW = 41.8kDa.

Specific Activity (Parent lot# WAB0496): As provided, this lot demonstrated 3.8% of maximum activity. Activated by phosphorylation with ROCKII (cat# 14-451).

Formulation: 1.07mg/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.

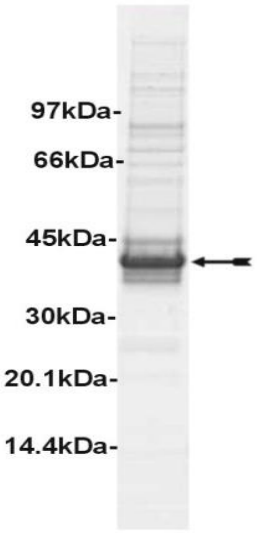
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Activation Assay: Unactive LIM Kinase 1 was activated using ROCKII (cat# 14-451), and the increased activity against cofilin 1 determined. The activation and subsequent assay is described on page two. Results of this assay are shown below.

Active ROCKII	Unactive LIM Kinase 1	Mean cpm	Comments
317ng	None	191	Background
None	13.9µg	292	Background
317ng	13.9µg	3646	Kinase activity

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, unactive.

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Kinase Assay Protocol

Stock Solutions:

1. **10 x Activation Buffer:** 500µM Tris/HCl pH7.5, 1mM EGTA, 1% 2-mercaptoethanol.
2. **ROCKII, active (Catalogue# 14-451):** Use at a final concentration of 0.1µM (0.00634mg/ml). Make a 0.127mg/ml stock. Add 2.5µl of stock per assay point.
3. **LIM Kinase 1, unactive:** Use at a final concentration of 6.67µM (0.279mg/ml). Add 13.0µl of stock per assay point.
4. **Magnesium/ATP cocktail:** (10 x stock) 1mM ATP and 100mM magnesium acetate. Add 5µl of stock per assay point.
5. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
6. **Enzyme Dilution Buffer:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA.
7. **Cofilin 1:** Use at a final concentration of 0.634mg/ml. Make a 3.17mg/ml stock. Add 2.5µl of stock per assay point.
8. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure:

Stage 1: *Activation of LIM Kinase 1 by ROCKII*

1. Add 5µl of 10 x activation buffer to a microcentrifuge tube.
2. Add 2.5µl of 0.127mg/ml **ROCKII, active**.
3. Add **13.0µl of 1.07mg/ml LIM Kinase 1, unactive**.
4. Add 24.5µl of dH_2O.
5. Add 5µl of magnesium/ATP cocktail.
6. Incubate at for 30 minutes at 30°C.
7. Stop the reaction by diluting 1/200, 1/400, 1/800 and 1/1600 with enzyme dilution buffer and storing on ice.

Stage 2: *Phosphorylation of cofilin 1 by LIM Kinase 1*

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of 3.17mg/ml **cofilin 1**.
3. Add **2.5µl (0.4-3.5ng) LIM Kinase 1, active** from **Stage One**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 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 QRVSFAKDIA SGMAYLHSMN
181 IIHRDLNSHN CLVRENKNVV VADFGLARLM 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 ggcagaaacc tgtcttgagg
  61 agctgcagca tcgacaggtc tccgggcgct ggctcactgg gctccccggc ctcccagcgc
 121 aaggacctgg gtcgctctga gtccctccgc gtagtctgcc ggccacaccg catcttccgg
 181 ccgtcggacc tcatccacgg ggaggtgctg ggcaagggct gcttcggcca ggctatcaag
 241 gtgacacacc gtgagacagg tgaggtgatg gtgatgaagg agctgatccg gttcgacgag
 301 gagacccaga ggacgttcct caaggaggtg aaggtcatgc gatgcctgga acaccccaac
 361 gtgctcaagt tcatcggggt gctctacaag gacaagaggc tcaacttcat cactgagtac
 421 atcaagggcg gcacgctccg gggcatcatc aagagcatgg acagccagta cccatggagc
 481 cagagagtga gctttgccaa ggacatcgca tcagggatgg cctacctcca ctccatgaac
 541 atcatccacc gagacctcaa ctcccacaac tgcctggtcc gcgagaacaa gaatgtggtg
 601 gtggctgact tcgggctggc gcgtctcatg gtggacgaga agactcagcc tgagggcctg
 661 cggagcctca agaagccaga ccgcaagaag cgctacaccg tggtgggcaa cccctactgg
 721 atggcacctg agatgatcaa cggccgcagc tatgatgaga aggtggatgt gttctccttt
 781 gggatcgtcc tgtgcgagat catcgggcgg gtgaacgcag accctgacta cctgccccgc
 841 accatggact ttggcctcaa cgtgcgagga ttcctggacc gctactgccc cccaaactgc
 901 cccccgagct tcttccccat caccgtgcgc tgttgcgatc tggaccccga gaagaggcca
 961 tcctttgtga agctggaaca ctggctggag accctccgca tgcacctggc cggccacctg
1021 ccactgggcc cacagctgga gcagctggac agaggtttct gggagaccta ccggcgcggc
1081 gagagcggat ga
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Reviewed and approved by site quality representative.