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Certificate of Analysis

MKK6/SKK3, unactive

(Recombinant enzyme expressed in *E.coli* cells)
Item # 14-304, 14-304-K, 14-304M
Parent Lot # 1604418

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 maltose binding protein tagged, recombinant MKK6 amino acids 4–end, expressed in *E.coli* cells. Purified using amylase agarose. Purity 99% by SDS-PAGE and Coomassie blue staining. MW = 80kDa.

Specific Activity (Parent lot# 1604418): As provided, this lot demonstrated 3% of maximum activity. Activated by phosphorylation with MEKK (cat# 14-196).

Formulation: 3.19mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.1% 2-mercapteothanol, 0.03% Brij-35, 20% glycerol. 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.

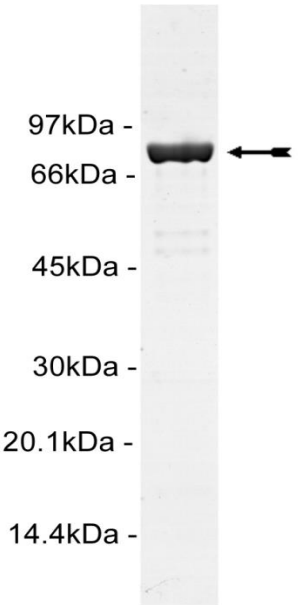
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Activation Assay: 4µM MKK6, unactive was activated using 200µg/ml MEKK (Catalogue# 14-196) which in turn was used to activate 2µM SAPK2a, and the increased activity against MBP determined. The activation and assay are described on pages two and three. Results of this assay are shown below.

Active MEKK	Unactive MKK6	Mean cpm	Comments
5µg	8.06µg	20658	Kinase activity
None	8.06µg	914	Background

MS Tryptic Fingerprint: Confirmed product identity as MKK6 with the translated sequence listed on page four



DS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of MKK6, unactive.

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Kinase Assay Protocol

Stock Solutions

1. **10 x Activation Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1% 2-mercaptoethanol, 1mM Na_3VO_4, 10mg/ml BSA.
2. **10 x Reaction Buffer:** 250mM Tris/HCl pH7.5, 1mM EGTA.
3. **Enzyme Dilution Buffer:** 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.1mM Na_3VO_4, 1mg/ml BSA.
4. **5 x Mg/ATP (Stages One and Two):** 50mM MgAc, 0.5mM ATP.
5. **MEKK:** Use at a final assay concentration of 200µg/ml. Prepare a 1mg/ml stock. Use 5µl of stock per assay point.
6. **MKK6 unactive:** Use at a final assay concentration of 4µM (0.322mg/ml). Prepare a 1mg/ml stock. Use 8µl of stock per assay point.
7. **SAPK2a unactive:** Use at a final assay concentration of 2µM (0.135mg/ml). Prepare a 0.675mg/ml stock. Use 5µl of stock per assay point.
8. **MBP:** Use at a final assay concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock. Use 2.5µl of stock per assay point.
9. **[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 One: *Activation of MKK6 by MEKK*

1. Add 2.5µl of 10 x activation buffer to a microcentrifuge tube.
2. Add 5µl of **MEKK**.
3. Add **8µl MKK6, unactive**.
4. Add 4.5µl of dH_2O.
5. Add 5µl of stage one Mg/ATP mixture. (In appropriate controls add dilution buffer to a final volume of 25µl).
6. Incubate for 1 hour at 30°C.
7. Stop the reaction by diluting the MKK6 250-1000 fold and storing on ice.

Stage Two: *Activation of SAPK2a by MKK6*

1. Add 2.5µl of 10 x activation buffer to a microcentrifuge tube.
2. Add 5µl of **SAPK2a unactive**.
3. Add **2.5µl** diluted **MKK6 (activated) from Stage One.**
4. Add 10µl of dH_2O.
5. Add 5µl of stage two Mg/ATP mixture.
6. Incubate at 30°C for 15 minutes
7. Stop reaction by immediately transferring 5µl into **Stage Three** reaction mixture.

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Stage Three: *Phosphorylation of MBP by SAPK2a*

1. Add 2.5µl of reaction buffer to a microcentrifuge tube.
2. Add 2.5µl of **MBP**.
3. Add 5µl of **activated SAPK2a from Stage Two.**
4. Add 5µl of dH_2O.
5. Add 10µl of the diluted [γ–^{33}P]ATP mixture.
6. Incubate for 15 minutes at 30°C.
7. Transfer a 20µl aliquot onto the centre of a 2cm x 2cm **P81** paper square.
8. Wash the assay squares twice for 5 minutes with 75mM phosphoric acid.
9. Wash the assay squares once for 2 minutes with acetone.
10. Transfer the assay squares to a vial and add 1ml scintillation cocktail.
11. Read in 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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MKK6 (4-end) Sequence Information

Protein human MKK6 (4–end)

Tags *N*-terminal maltose binding protein

Native sequence S396 of the recombinant protein is equivalent to S4 of human MKK6

Accession number EMBL U39657

Recombinant MKK6 amino acid sequence:

```
  1 MKTEEGKLVI WINGDKGYNG LAEVGKKFEK DTGIKVTVEH PDKLEEKFPQ VAATGDGPDI
 61 IFWAHDRFGG YAQSGLLAEI TPDKAFQDKL YPFTWDAVRY NGKLIAYPIA VEALSLIYNK
121 DLLPNPPKTW EEIPALDKEL KAKGKSALMF NLQEPYFTWP LIAADGGYAF KYENGKYDIK
181 DVGVDNAGAK AGLTFLVDLI KNKHMNADTD YSIAEAAFNK GETAMTINGP WAWSNIDTSK
241 VNYGVTVLPT FKGQPSKPFV GVLSAGINAA SPNKELAKEF LENYLLTDEG LEAVNKDKPL
301 GAVALKSYEE ELAKDPRIAA TMENAQKGEI MPNIPQMSAF WYAVRTAVIN AASGRQTVDE
361 ALKDAQTNSS SNNNNNNNNN NLGIEGRISE FGSSRSKGKK RNPGLKIPKE AFEQPQTSST
421 PPRDLDSKAC ISIGNQNFEV KADDLEPIME LGRGAYGVVE KMRHVPSGQI MAVKRIRATV
481 NSQEQKRLLM DLDISMRTVD CPFTVTFYGA LFREGDVWIC MELMDTSLDK FYKQVIDKGQ
541 TIPEDILGKI AVSIVKALEH LHSKLSVIHR DVKPSNVLIN ALGQVKMCDF GISGYLVDSV
601 AKTIDAGCKP YMAPERINPE LNQKGYSVKS DIWSLGITMI ELAILRFPYD SWGTPFQQLK
661 QVVEEPSPQL PADKFSAEFV DFTSQCLKKN SKERPTYPEL MQHPFFTLHE SKGTDVASFV
721 KLILGD
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Recombinant MKK6 nucleotide sequence:

```
   1 atgaaaactg aagaaggtaa actggtaatc tggattaacg gcgataaagg ctataacggt
  61 ctcgctgaag tcggtaagaa attcgagaaa gataccggaa ttaaagtcac cgttgagcat
 121 ccggataaac tggaagagaa attcccacag gttgcggcaa ctggcgatgg ccctgacatt
 181 atcttctggg cacacgaccg ctttggtggc tacgctcaat ctggcctgtt ggctgaaatc
 241 accccggaca aagcgttcca ggacaagctg tatccgttta cctgggatgc cgtacgttac
 301 aacggcaagc tgattgctta cccgatcgct gttgaagcgt tatcgctgat ttataacaaa
 361 gatctgctgc cgaacccgcc aaaaacctgg gaagagatcc cggcgctgga taaagaactg
 421 aaagcgaaag gtaagagcgc gctgatgttc aacctgcaag aaccgtactt cacctggccg
 481 ctgattgctg ctgacggggg ttatgcgttc aagtatgaaa acggcaagta cgacattaaa
 541 gacgtgggcg tggataacgc tggcgcgaaa gcgggtctga ccttcctggt tgacctgatt
 601 aaaaacaaac acatgaatgc agacaccgat tactccatcg cagaagctgc ctttaataaa
 661 ggcgaaacag cgatgaccat caacggcccg tgggcatggt ccaacatcga caccagcaaa
 721 gtgaattatg gtgtaacggt actgccgacc ttcaagggtc aaccatccaa accgttcgtt
 781 ggcgtgctga gcgcaggtat taacgccgcc agtccgaaca aagagctggc aaaagagttc
 841 ctcgaaaact atctgctgac tgatgaaggt ctggaagcgg ttaataaaga caaaccgctg
 901 ggtgccgtag cgctgaagtc ttacgaggaa gagttggcga aagatccacg tattgccgcc
 961 accatggaaa acgcccagaa aggtgaaatc atgccgaaca tcccgcagat gtccgctttc
1021 tggtatgccg tgcgtactgc ggtgatcaac gccgccagcg gtcgtcagac tgtcgatgaa
1081 gccctgaaag acgcgcagac taattcgagc tcgaacaaca acaacaataa caataacaac
1141 aacctcggga tcgagggaag gatttcagaa ttcggatcct ctagatcgaa aggcaagaag
1201 cgaaaccctg gccttaaaat tccaaaagaa gcatttgaac aacctcagac cagttccaca
1261 ccacctcgag atttagactc caaggcttgc atttctattg gaaatcagaa ctttgaggtg
1321 aaggcagatg acctggagcc tataatggaa ctgggacgag gtgcgtacgg ggtggtggag
1381 aagatgcggc acgtgcccag cgggcagatc atggcagtga agcggatccg agccacagta
1441 aatagccagg aacagaaacg gctactgatg gatttggata tttccatgag gacggtggac
1501 tgtccattca ctgtcacctt ttatggcgca ctgtttcggg agggtgatgt gtggatctgc
1561 atggagctca tggatacatc actagataaa ttctacaaac aagttattga taaaggccag
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1621 acaattccag aggacatctt agggaaaata gcagtttcta ttgtaaaagc attagaacat
1681 ttacatagta agctgtctgt cattcacaga gacgtcaagc cttctaatgt actcatcaat
1741 gctctcggtc aagtgaagat gtgcgatttt ggaatcagtg gctacttggt ggactctgtt
1801 gctaaaacaa ttgatgcagg ttgcaaacca tacatggccc ctgaaagaat aaacccagag
1861 ctcaaccaga agggatacag tgtgaagtct gacatttgga gtctgggcat cacgatgatt
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Reviewed and approved by site quality representative.