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Certificate of Analysis

MKK6/SKK3 (S599D,T603D), active

(Recombinant enzyme expressed in *E.coli* cells)
Item # 14-537, 14-537-K, 14-537M
Parent Lot # 30938U

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 MBP tagged, recombinant human MKK6/SKK3 residues 4–end containing S599D and T603D mutations. Expressed in *E.coli* cells. Purified using amylose agarose. Purity 88.4% by SDS-PAGE and Coomassie staining. MW = 80.6kDa.

Specific Activity (Parent lot# 30938U): 1431U/mg, where one unit of MKK6/SKK3 activity is defined as the amount of MKK6/SKK3 which activates 2µM p38α/SAPK2a, unactive by 1 Unit per minute using 100µM ATP at 30°C. One unit of p38α/SAPK2a activity is defined as 1nmole of phosphate incorporated into 0.33mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 3.74mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.03% Brij-35, 0.1mM EGTA, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol, 270mM sucrose. Frozen solution.

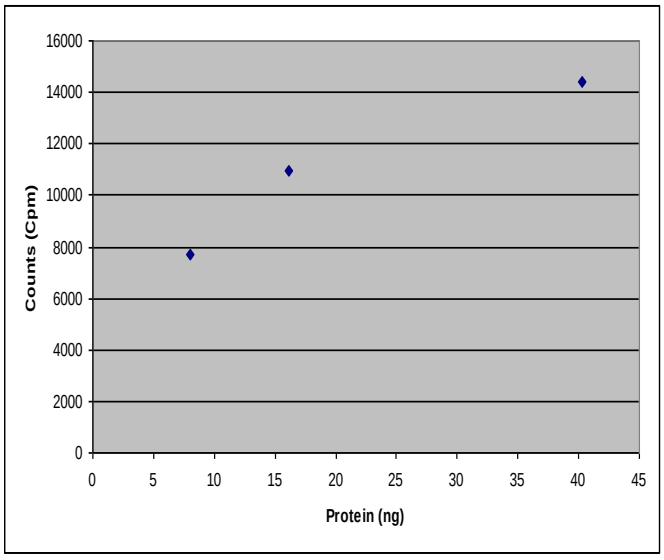
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

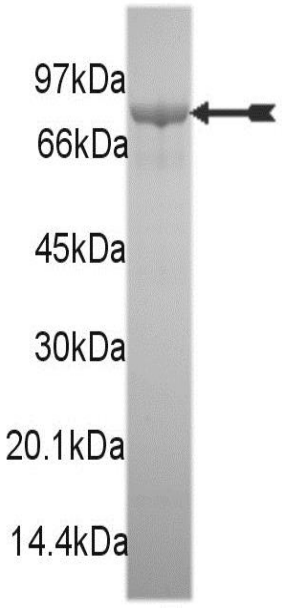
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 8–40ng of this lot of enzyme phosphorylated 2µM unactive p38α/SAPK2a, 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 MKK6/SKK3 with the translated sequence listed on page three and four.



SDS-PAGE and Coomassie Stain: Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of MKK6 /SKK3, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Activation Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na_3VO_4, 1% 2-mercaptoethanol, 10mg/ml BSA.
2. **10 x Reaction Buffer:** 250mM Tris/HCl pH7.5, 1mM EGTA.
3. **SAPK2a unactive:** Use a final assay concentration of 0.28mg/ml (2µM). Make up a 2.8mg/ml stock. Use 2.5µl of stock per assay point.
4. **Myelin Basic Protein (MBP):** Use a final assay concentration of 0.33mg/ml. Make up a 3.33mg/ml stock. Use 2.5µl of stock per assay point.
5. **MKK6/SKK3, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na_3VO_4, 1mg/ml BSA. Use 8–40ng per assay point.
6. **Stage One 5 x Mg/ATP:** 50mM magnesium acetate, 0.5mM ATP.
7. **[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 p38α/SAPK2a*

1. Add 5µl of 5 x activation buffer.
2. Add 2.5µl of **SAPK2a unactive**.
3. Add **2.5µl (8–40ng) of MKK6 /SKK3 active**.
4. Add 10µl of dH_2O.
5. Add 5µl of stage one Mg/ATP cocktail.
6. Incubate for 15 minutes at 30°C. Immediately transfer **5µl** into **Stage Two**.

Stage Two: *Phosphorylation of MBP by activated p38α/SAPK2a*

1. Add 2.5µl of 10 x reaction buffer.
2. Add 2.5µl of **MBP**.
3. Add **5µl** of **Stage One** reaction product.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P] ATP mixture.
6. Incubate for 15 minutes at 30°C.
7. Transfer a 20µl aliquot onto the centre of a 2 cm x 2 cm **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 scintillation vial and add 1ml of scintillation cocktail.
11. 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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MKK6/SKK3 (S599D,T603D) Sequence Information

Protein human MKK6/SKK3 (S599D,T603D)

Tags N-terminal maltose-binding protein (MBP)

Native sequence S396 of the recombinant protein is equivalent to S4 of human MKK6/SKK3 (S599D,T603D). S599 and T603 of the recombinant sequence have been mutated to aspartate to provide constitutive activation of the enzyme.

Accession number EMBL U39657

Recombinant MKK6/SKK3 (S599D, T603D) 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 GISGYLVDDV
 601 AKDIDAGCKP YMAPERINPE LNQKGYSVKS DIWSLGITMI ELAILRFPYD SWGTPFQQLK
 661 QVVEEPSPQL PADKFSAEFV DFTSQCLKKN SKERPTYPEL MQHPFFTLHE SKGTDVASFV
 721 KLILGD
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Recombinant MKK6/SKK3 (S599D, T603D) nucleotide sequence:

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   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
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1561 atggagctca tggatacatc actagataaa ttctacaaac aagttattga taaaggccag
1621 acaattccag aggacatctt agggaaaata gcagtttcta ttgtaaaagc attagaacat
1681 ttacatagta agctgtctgt cattcacaga gacgtcaagc cttctaatgt actcatcaat
1741 gctctcggtc aagtgaagat gtgcgatttt ggaatcagtg gctacttggt ggacgatgtt
1801 gctaaagata ttgatgcagg ttgcaaacca tacatggccc ctgaaagaat aaacccagag
1861 ctcaaccaga agggatacag tgtgaagtct gacatttgga gtctgggcat cacgatgatt
1921 gagttggcca tccttcgatt tccctatgat tcatggggaa ctccatttca gcagctcaaa
1981 caggtggtag aggagccatc gccacaactc ccagcagaca agttctctgc agagtttgtt
2041 gactttacct cacagtgctt aaagaagaat tccaaagaac ggcctacata cccagagcta
2101 atgcaacatc catttttcac cctacatgaa tccaaaggaa cagatgtggc atcttttgta
2161 aaactgattc ttggagacta a
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Reviewed and approved by site quality representative.