

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, active activity is defined as the amount of MKK6/SKK3 which activates 2 μ M p38 α /SAPK2 α , inactive by 1 Unit per minute using 100 μ M ATP at 30°C. One unit of p38 α /SAPK2 α 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 100 μ M.

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.

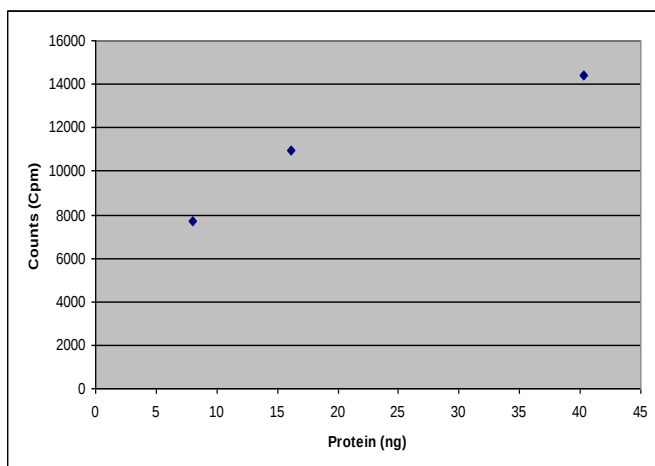
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

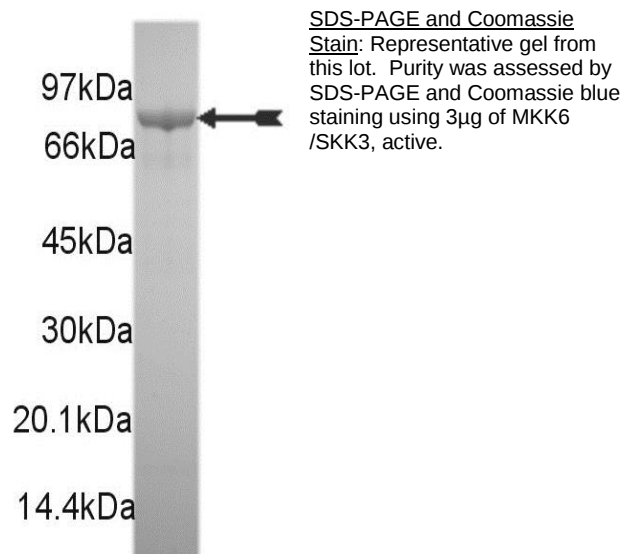
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 8–40ng of this lot of enzyme phosphorylated 2 μ M inactive p38 α /SAPK2 α , 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.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Activation Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na₃VO₄, 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 Na₃VO₄, 1mg/ml BSA. Use 8–40ng per assay point.
6. **Stage One 5 x Mg/ATP:** 50mM magnesium acetate, 0.5mM ATP.
7. **[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

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 dH₂O.
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 dH₂O.
5. Add 10μl of diluted [γ-³³P] 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.

Certificate of Analysis

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:

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1 MKTEEGKLVI WINGDKGYNG LAEVGKKFEK DTGIKVTVEH PDKLEEKFPQ VAATGDGPD
61 IFWAHDFRFG YAQSGLLAEI TPDKAFQDKL YPFTWDAVRY NGKLIAYPIA VEALSIIYNK
121 DLLPNPPKTW EEIPALDKEL KAKGKSALMF NLQEPYFTWP LIAADGGYAF KYENGKYDIK
181 DVGVDNAGAK AGLTFLVDLI KNKHMNADTD YSIAEAAFNK GETAMTINGP WAWSNIDTSK
241 VNYGVTVLPT FKGQPSKPFV GVLSAGINAA SPNKELAKEF LENYLLTDEG LEAVNKDKPL
301 GAVALKSIEE ELAKDPRIAA TMENAQKGEI MPNIPQMSAF WYAVRTAVIN AASGRQTVDE
361 ALKDAQTNSS SNNNNNNNNN NLGIEGRISE FGSSRSKGKK RNPGLKIPKE AFEQPQTSST
421 PPRDLDSKAC ISIGNQNFV KADDLEPIME LGRGAYGVVE KMRHVPSGQI MAVKRIRATV
481 NSQEQKRLLM DLDISMRTVD CPFTVTFYGA LFREGDVWIC MELMDTSLDK FYKQVIDKGQ
541 TIPEDILGKI AVSIVKALEH LHSKLSVIHR DVKPSNVLIN ALGQVKMCDG GISGYLVDDV
601 AKDIDAGCKP YMAPERINPE LNQKGYSVKS DIWSLGITMI ELAILRFPYD SWGTPFQQLK
661 QVVEEPSQPL 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 tcggtgaagaa attcgagaaa gataccggaa ttaaagtcac cgttgagcat
121 ccggataaac tggaagagaa attcccacag gttgcggcaa ctggcgatgg ccctgacatt
181 atcttctggg cacacgaccg ctttgggtggc tacgctcaat ctggcctggt 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 gcggtctga ccttcctggt tgacctgatt
601 aaaaacaaac acatgaatgc agacaccgat tactccatcg cagaagctgc ctttaataaa
661 ggcgaaacag cgatgaccat caacggcccc tgggcatggt ccaacatcga caccagcaaa
721 gtgaattatg gtgtaacggg actgccgacc ttcaagggtc aaccatccaa accgttcgtt
781 ggcgtgctga gcgcaggtat taacgccgcc agtccgaaca aagagctggc aaaagagttc
841 ctcgaaaact atctgctgac tgatgaaggt ctggaagcgg ttaataaaga caaacgcgtg
901 ggtgccgtag cgctgaagtc ttacgaggaa gattggcgca aagatccacg tattgccgcc
961 accatggaaa acgccagaaa aggtgaaatc atgccgaaca tcccgcagat gtccgctttc
1021 tggatatgcc tgcgactgct ggtgatcaac gccgccagcg gtcgtcagac tgtcgatgaa
1081 gccctgaaag acgcgagac taattcgagc tcgaacaaca acaacaataa caataacaac
1141 aacctcgga tgcaggggaa gatttcagaa ttcggatcct ctagatcgaa aggcaagaag
1201 cgaaacctg gccttaaaat tccaaaagaa gcatttgaac aacctcagac cagttccaca
1261 ccacctcgag atttagactc caaggcttgc atttctattg gaaatcagaa ctttgagggtg
1321 aaggcagatg acctggagcc tataatggaa ctgggacgag gtgcgtacgg ggtgggtggag
1381 aagatgcggc acgtgccag cgggcagatc atggcagtg agcggatccg agccacagta
1441 aatagccagg aacagaaacg gctactgatg gatttgataa tttccatgag gacgggtggac
1501 tgtccattca ctgtcacctt ttatggcgca ctgtttcggg aggggtgatgt gtggatctgc

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Certificate of Analysis

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1561 atggagctca tggatacatc actagataaa ttctacaaac aagttattga taaaggccag
1621 acaattccag aggacatctt agggaaaata gcagtttcta ttgtaaaagc attagaacat
1681 ttacatagta agctgtctgt cattcacaga gacgtcaagc cttctaattgt actcatcaat
1741 gctctcggtc aagtgaagat gtgcgatttt ggaatcagtg gctacttggt ggacgatgtt
1801 gctaaagata ttgatgcagg ttgcaaacca tacatggccc ctgaaagaat aaaccagag
1861 ctcaaccaga agggatacag tgtgaagtct gacatttgga gtctgggcat cacgatgatt
1921 gagttggcca tccttcgatt tccctatgat tcatggggaa ctccatttca gcagctcaaa
1981 caggtggtag aggagccatc gccacaactc ccagcagaca agttctctgc agagtttggt
2041 gactttacct cacagtgcct aaagaagaat tccaaagaac ggctacata cccagagcta
2101 atgcaacatc cttttttcac cctacatgaa tccaaaggaa cagatgtggc atcttttgta
2161 aaactgattc ttggagacta a
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