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Certificate of Analysis

MAPKAP Kinase 3, unactive

(Recombinant enzyme expressed in *E.coli* cells)
Item # 14-586, 14-586-K, 14-586M
Parent Lot # 1952459

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, GST-tagged, recombinant human MAPKAP Kinase 3, amino acids 2–end, expressed in *E.coli* cells. Purified using glutathione agarose. Purity 81.1% by SDS-PAGE and Coomassie blue staining. MW=69.8kDa.

Specific Activity (Parent lot# 1952459): As provided, this lot demonstrated <1% of maximum activity. Activated by phosphorylation with SAPK2a (cat# 14-587).

Formulation: 3.177mg/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

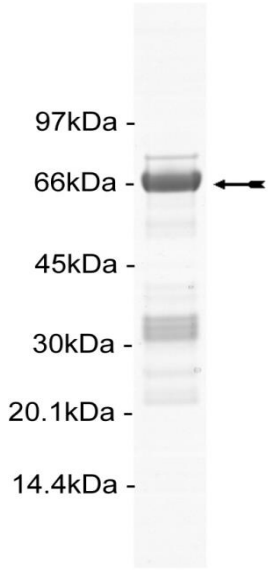
Quality Control Testing

Activation Assay: 4µM unactive MAPKAP Kinase 3 was activated using 0.2µM SAPK2a (cat# 14-587) diluted 200–2500 fold, and the increased activity against (KKLNRTLSVA) (cat# 12-240) determined. The activation and assay are described on page two. Results of this assay are shown below

Active SAPK2a	Unactive MAPKAP Kinase 3	Mean cpm	Comments
0.218µg	None	74	Background
none	3.5µg	115	Background
0.218µg	3.5µg	4834	Kinase activity

MS Tryptic Fingerprint: Confirmed identity as MAPKAP Kinase 3 with the translated sequence listed on page three

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



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Kinase Activation Assay Protocol

Stock Solutions:

1. **10 x Activation Buffer:** 50mM Tris/HCl pH7.5, 1mM EGTA, 1% 2-mercaptoethanol.
2. **20 x Assay Buffer:** 1M Na-β-glycerophosphate, 2mM EGTA, pH7.5.
3. **Enzyme Dilution Buffer:** 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 1% 2-mercaptoethanol, 1mg/ml BSA.
4. **MAPKAP Kinase 3, unactive:** Use at a final assay concentration of 4µM (0.28mg/ml). Prepare a 1.4mg/ml stock and add 5µl of stock per assay point.
5. **SAPK2a, active (Catalogue# 14-587):** Use at a final assay concentration of 0.2µM (0.009mg/ml). Prepare a 0.09mg/ml stock and add 2.5µl of stock per assay point.
6. **Stage One 5 x Mg/ATP:** 50mM magnesium acetate, 0.5mM ATP.
7. **(KKLNRTLSVA):** Use at a final concentration of 30µM. Make up a 300µM stock. Use 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 268cpm/pmol as required.)

Assay Procedure:

Stage One: *Activation of MAPKAP Kinase 3 by SAPK2a*

1. Add 2.5µl of 10 x activation buffer to a microcentrifuge tube.
2. Add **5µl of MAPKAP Kinase 3, unactive**.
3. Add 2.5µl of **SAPK2a, active**.
4. Add 10µl of dH2O.
5. Add 5µl of stage one Mg/ATP mixture.
6. Incubate for 15 minutes at 30°C.
7. Stop reaction by diluting the tubes 200–2500 fold and incubating on ice.

Stage Two: *Phosphorylation of KKLNRTLSVA by MAPKAP Kinase 3*

1. Add 1.25µl of 20 x reaction buffer to a microcentrifuge tube.
2. Add 2.5 µl of **(KKLNRTLSVA)**.
3. Add **2.5µl (0.2–1.4ng) MAPKAP Kinase3, active** from **Stage One**.
4. Add 8.75µl dH_20
5. Add 10µl of [γ-^{33}P]ATP mix.
6. Incubate for 10 minutes at 30°C.
7. Slowly transfer 20µl onto the centre of a 2 cm x 2 cm **P81** paper.
8. Wash assay squares twice for 5 minutes with 75mM phosphoric acid.
9. Wash assay squares once for 2 minutes with acetone.
10. Transfer assay squares to scintillation vials 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 and 1µl of 30% phosphoric acid (background control).

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MAPKAP Kinase 3 Sequence Information

Protein Human MAPKAP Kinase 3

Tags *N*-terminal GST

Native sequence D232 of the recombinant protein is equivalent to D2 of human MAPKAP Kinase 3

Accession number GenBank NM_004635

Recombinant MAPKAP Kinase 3 amino acid sequence:

```
  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPLG SDGETAEEQG
241 GPVPPPVAPG GPGLGGAPGG RREPKKYAVT DDYQLSKQVL GLGVNGKVLE CFHRRTGQKC
301 ALKLLYDSPK ARQEVDHHWQ ASGGPHIVCI LDVYENMHHG KRCLLIIMEC MEGGELFSRI
361 QERGDQAFTE REAAEIMRDI GTAIQFLHSH NIAHRDVKPE NLLYTSKEKD AVLKLTDFGF
421 AKETTQNALQ TPCYTPYYVA PEVLGPEKYD KSCDMWSLGV IMYILLCGFP PFYSNTGQAI
481 SPGMKRRIRL GQYGFPNPEW SEVSEDAKQL IRLLLKTDPT ERLTITQFMN HPWINQSMVV
541 PQTPLHTARV LQEDKDHWDE VKEEMTSALA TMRVDYDQVK IKDLKTSNNR LLNKRRKKQA
601 GSSSASQGCN NQ
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Recombinant MAPKAP Kinase 3 nucleotide sequence:

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   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
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 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
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```
1741 atcaaggacc tgaagacctc taacaaccgg ctcctcaaca agaggagaaa aaagcaggca
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Reviewed and approved by site quality representative.