

Certificate of Analysis

MAPKAP Kinase 3, active

(Recombinant enzyme expressed in *E.coli* cells)

Item # 14-585, 14-585-K, 14-585M

Parent Lot # D8BN029U

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. Activated using SAPK2a (cat# 14-587) and repurified using glutathione agarose. Purity 97.2% by SDS-PAGE and Coomassie blue staining. MW = 69.8kDa.

Specific Activity (Parent lot# D8BN029U): 3540U/mg, where one unit of MAPKAP Kinase 3, active activity is defined as 1nmol phosphate incorporated into 30μM (KKLNRTLVA) (cat# 12-240) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 1.904mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.03% Brij-35, 0.1mM EGTA, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

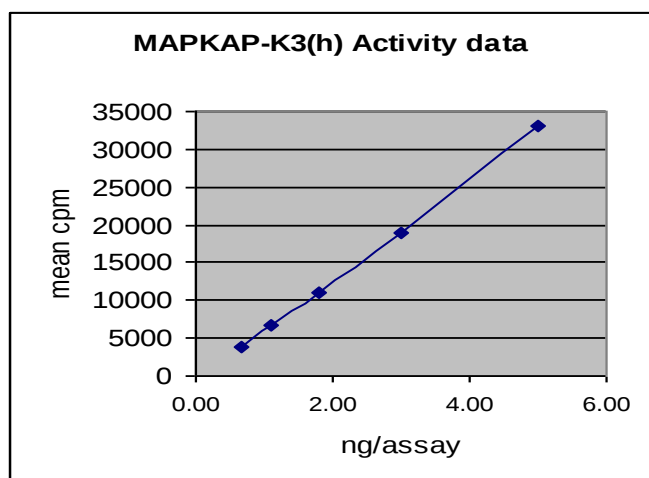
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

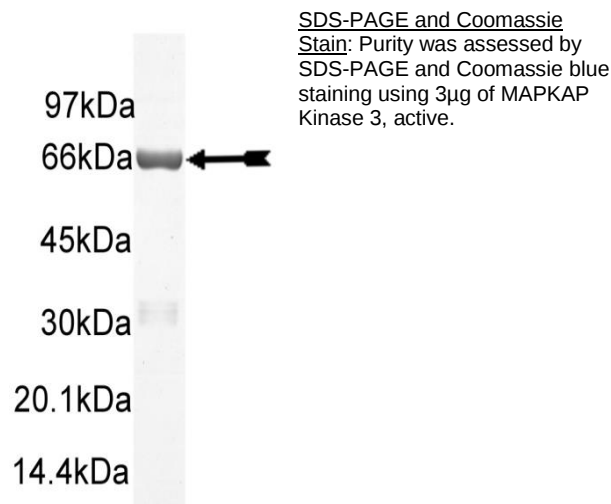
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.66–5.00ng of this lot of enzyme phosphorylated 30μM (KKLNRTLVA) 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 MAPKAP Kinase 3 with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **20 x Reaction Buffer:** 1M Na- β -glycerophosphate, 2mM EGTA, pH7.5.
2. **(KKLNRTLVA):** Use at a final concentration of 30 μ M. Make up a 300 μ M stock. Use 2.5 μ l of stock per assay point.
3. **MAPKAP Kinase 3, active:** Dilute with 20mM MOPS, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.66–5.00ng per assay point.
4. **[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 (96 well plate format):

1. Add 1.25 μ l of 20 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(KKLNRTLVA)**.
3. Add **2.5 μ l (0.66–5.00ng) MAPKAP Kinase 3, active**.
4. Add 8.75 μ l dH₂O
5. Add 10 μ l of [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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MAPKAP Kinase 3 Sequence Information

<u>Protein</u>	Human MAPKAP Kinase 3
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	D232 of the recombinant protein is equivalent to D2 of human MAPKAP Kinase 3
<u>Accession number</u>	GenBank NM_004635

Recombinant MAPKAP Kinase 3 amino acid sequence:

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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  ARQEVDDHWQ  ASGGPHIVCI  LDVYENMHHG  KRCLLIIMEC  MEGGELFSRI
361  QERGDQAFTE  REAAEIMRDI  GTAIQFLHSH  NIAHRDVKPE  NLLYTSKEKD  AVLKLTDFGF
421  AKETTQNALQ  TPCYTPYYVA  PEVLGPEKYD  KSCDMWSLGV  IMYILLCGFP  PFYSNTGQAI
481  SPGMKRRIRL  GQYGFPNPEW  SEVEDAKQL  IRLLLKTDPT  ERLTITQFMN  HPWINQSMVV
541  PQTPLHTARV  LQEDKDHWE  VKEEMTSALA  TMRVDYDQVK  IKDLKTSNNR  LLNKRKKQA
601  GSSSASQGCN  NQ

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Recombinant MAPKAP Kinase 3 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgtagt  agcgcatga  aggtgataaa
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1681  gtcaaggagg  agatgaccag  tgccttggcc  actatgcggg  tagactacga  ccaggtgaag

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1741 atcaaggacc tgaagacctc taacaaccgg ctctcaaca agaggagaaa aaagcaggca
1801 ggcagctcct ctgcctcaca gggctgcaac aaccagtag

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