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Certificate of Analysis

MAPKAP Kinase 2, unactive

(Recombinant enzyme expressed in *E.coli* cells)
Item # 14-349, 14-349-K, 14-349M
Parent Lot # 1691879

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 2, amino acids 46–end, expressed in *E.coli* cells. Purified using glutathione agarose. Purity 78.7% by SDS-PAGE and Coomassie blue staining. MW=70.2kDa.

Specific Activity (Parent lot# 1691879): As provided, this lot demonstrated <1% of maximum activity. Activated by phosphorylation with MAP Kinase 2 (cat# 14-173.)

Formulation: 0.914mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol, 5mM DTT. Liquid at -20°C.

Storage and Stability: On receipt of material store at -20°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.

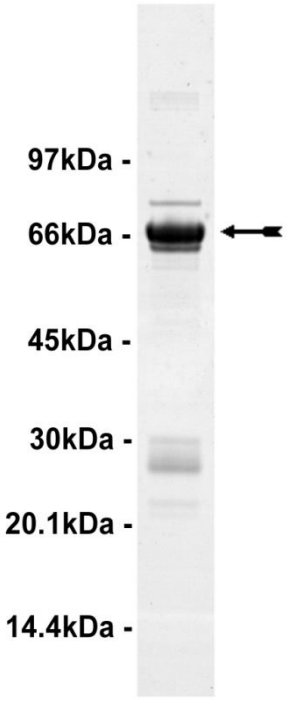
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Activation Assay: 4µM unactive MAPKAP Kinase 2 was activated using 0.2µM active MAP Kinase 2 (cat# 14-173) diluted 500–1000 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 MAP Kinase 2	Unactive MAPKAP Kinase 2	Mean cpm	Comments
0.34µg	7µg	7480	Kinase activity
none	7µg	640	Background

MS Tryptic Fingerprint: Confirmed identity as MAPKAP Kinase 2 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 2, unactive.

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Kinase Assay Protocol

Stock Solutions:

1. **10 x Activation Buffer:** 50mM Tris/HCl pH7.5, 1mM EGTA, 1% 2-mercaptoethanol.
2. **20 x Reaction Buffer:** 1M Na-β-glycerophosphate, 2mM EGTA.
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 2, 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. **MAP Kinase 2, active (Catalogue # 14-173):** Use at a final assay concentration of 0.2µM (0.014mg/ml). Prepare a 0.14mg/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. **[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.)
8. **MAPKAP Kinase 2 substrate peptide (KKLNRTLSVA) (Catalogue# 12-240):** Use at a final assay concentration of 30µM. Prepare a 300µM stock. Add 2.5µl of stock per assay point.

Assay Protocol:

Stage One: *Activation of MAPKAP Kinase 2, by MAP Kinase 2:*

1. Add 2.5µl of 10 x activation buffer to a microcentrifuge tube.
2. Add 5µl (7µg) of **MAPKAP Kinase 2, unactive**.
3. Add 2.5µl (0.34µg) of **MAP Kinase 2, active**.
4. Add 10µl of dH_2O.
5. Add 5µl of stage one Mg/ATP mixture.
6. Incubate for 30 minutes at 30°C.
7. Stop reaction by diluting the tubes 500–1000 fold and incubating on ice.

Stage Two: *Phosphorylation* of KKLNRTLSVA *substrate peptide with MAPKAP Kinase2* (96 well plate format):

1. Add 1.25µl of reaction buffer per assay to wells.
2. Add 2.5µl of substrate peptide **(KKLNRTLSVA)**.
3. Add 2.5µl of diluted MAPKAP Kinase 2 from **Stage One**.
4. Add 8.7µl of dH_2O.
5. Add 10µl of the diluted [γ-^{33}P] ATP.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Slowly transfer 10µl onto appropriate area of a **P30 Filtermat**.
9. Wash filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash filtermat once for 2 minutes with methanol.
11. Transfer filtermat to a sealable plastic bag and add 4ml scintillation cocktail.
12. Read in scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all appropriate assay components plus 1µl of 30% phosphoric acid enzyme (background control).

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MAPKAP Kinase 2, Sequence Information

Protein human MAPKAP Kinase 2

Tags *N*-Terminal GST

Native sequence F243 of the recombinant protein is equivalent to F46 of human MAPKAP Kinase 2

Accession number GenBank NM_032960. The recombinant sequence is D116H (native co-ordinates) with respect to GenBank NM_032960. This conflict is reported in SWISSPROT P49137. The cDNA also contains the translational conflict A399G, and a c-myc epitope at the C-terminus.

Recombinant MAPKAP Kinase 2 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 LVPRGSPGIS GGGGGILEAT
241 MEFHVKSGLQ IKKNAIIDDY KVTSQVLGLG INGKVLQIFN KRTQEKFALK MLQDCPKARR
301 EVELHWRASQ CPHIVRIVDV YENLYAGRKC LLIVMECLDG GELFSRIQDR GDQAFTEREA
361 SEIMKSIGEA IQYLHSINIA HRDVKPENLL YTSKRPNAIL KLTDFGFAKE TTSHNSLTTP
421 CYTPYYVAPE VLGPEKYDKS CDMWSLGVIM YILLCGYPPF YSNHGLAISP GMKTRIRMGQ
481 YEFPNPEWSE VSEEVKMLIR NLLKTEPTQR MTITEFMNHP WIMQSTKVPQ TPLHTSRVLK
541 EDKERWEDVK EEMTSALATM RVDYEQIKIK KIEDASNPLL LKRRKKARAL EAAALGHMEQ
601 KLISEEDLK
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Recombinant MAPKAP Kinase 2 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
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggttccgc gtggatcccc gggaatttcc ggtggtggtg gtggaattct agaggccacc
 721 atggagttcc acgtcaagtc cggcctgcag atcaagaaga acgccatcat cgatgactac
 781 aaggtcacca gccaggtcct ggggctgggc atcaacggca aagttttgca gatcttcaac
 841 aagaggaccc aggagaaatt cgccctcaaa atgcttcagg actgccccaa ggcccgcagg
 901 gaggtggagc tgcactggcg ggcctcccag tgcccgcaca tcgtacggat cgtggatgtg
 961 tacgagaatc tgtacgcagg gaggaagtgc ctgctgattg tcatggaatg tttggacggt
1021 ggagaactct ttagccgaat ccaggatcga ggagaccagg cattcacaga aagagaagca
1081 tccgaaatca tgaagagcat cggtgaggcc atccagtatc tgcattcaat caacattgcc
1141 catcgggatg tcaagcctga gaatctctta tacacctcca aaaggcccaa cgccatcctg
1201 aaactcactg actttggctt tgccaaggaa accaccagcc acaactcttt gaccactcct
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1381 tactccaacc acggccttgc catctctccg ggcatgaaga ctcgcatccg aatgggccag
1441 tatgaatttc ccaacccaga atggtcagaa gtatcagagg aagtgaagat gctcattcgg
1501 aatctgctga aaacagagcc cacccagaga atgaccatca ccgagtttat gaaccaccct
1561 tggatcatgc aatcaacaaa ggtccctcaa accccactgc acaccagccg ggtcctgaag
1621 gaggacaagg agcggtggga ggatgtcaag gaggagatga ccagtgcctt ggccacaatg
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1681 cgcgttgact acgagcagat caagataaaa aagattgaag atgcatccaa ccctctgctg
1741 ctgaagaggc ggaagaaagc tcgggccctg gaggctgcgg ctctgggcca catggagcag
1801 aagctgatca gcgaggagga cctgaagtga
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Reviewed and approved by site quality representative.