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Certificate of Analysis

MAPKAP Kinase 2, active

(Recombinant enzyme expressed in *E.coli* cells)
Item # 14-337, 14-337-K, 14-337M
Parent Lot # WAE0265

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. Activated using MAP Kinase 2 and repurified using Q-sepharose. Purity 84% by SDS-PAGE and Coomassie blue staining. MW = 70.2kDa.

Specific Activity (Parent lot# WAE0265): 3002U/mg, where one unit of MAPKAP Kinase 2, active activity is defined as 1nmol phosphate incorporated into 30µM substrate peptide (KKLNRTLSVA, cat# 12-240) per minute at 30°C with a final ATP concentration of 100µM.

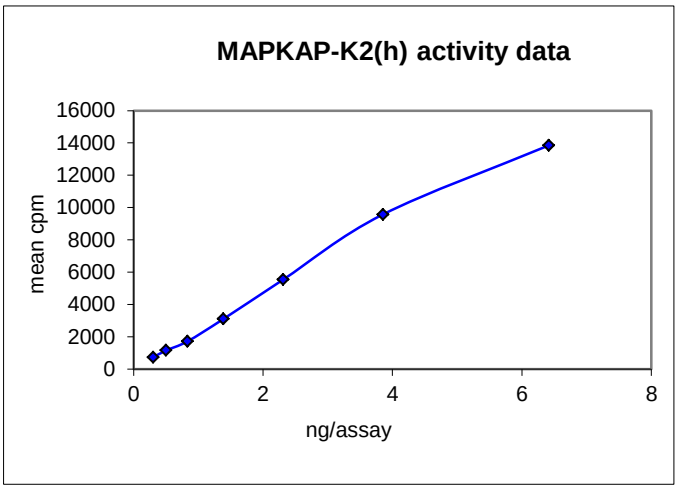
Formulation: 0.77mg/ml of enzyme in 50mM Tris/HCl, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol, 5mM dithiothreitol. Liquid at -20°C.

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.

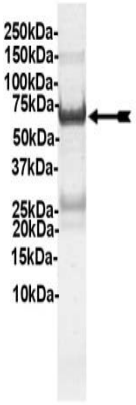
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.30–6.42ng of this lot of enzyme phosphorylated 30µM substrate peptide (KKLNRTLSVA) 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 product identity as MAPKAP Kinase 2 with the translated native 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, active.

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Kinase Assay Protocol

Stock Solutions:

1. **20 x Reaction Buffer:** 1M Na-β-glycerol phosphate, 2mM EGTA pH7.5.
2. **MAPKAP Kinase 2 substrate peptide (KKLNRTLSVA Catalogue# 12-240):** Use at a final assay concentration of 30µM. Make a 300µM stock. Add 2.5µl of stock per assay point.
3. **MAPKAP Kinase 2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.30–6.42ng per assay point.
4. **[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 (96 well plate format):

1. Add 5µl of 20 x reaction buffer per well.
2. Add 2.5µl of substrate peptide **(KKLNRTLSVA)**.
3. Add **2.5µl (0.30–6.42ng) MAPKAP-K2, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 50mM 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 1µl of 30% phosphoric acid.

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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:

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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 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
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 661 ctggttccgc gtggatcccc gggaatttcc ggtggtggtg gtggaattct agaggccacc
 721 atggagttcc acgtcaagtc cggcctgcag atcaagaaga acgccatcat cgatgactac
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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
1261 tgttatacac cgtactatgt ggctccagaa gtgctgggtc cagagaagta tgacaagtcc
1321 tgtgacatgt ggtccctggg tgtcatcatg tacatcctgc tgtgtgggta tccccccttc
1381 tactccaacc acggccttgc catctctccg ggcatgaaga ctcgcatccg aatgggccag
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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
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.