Certificate of Analysis
MAPK14 (p38 alpha), Inactive, 100 µg

Mitogen Activated Protein (MAP) Kinase 14, Inactive, GST-tagged

Part Number: PV3305
Lot Number: 2725248D
Immediate Storage: -80°C
Shipping Conditions: dry ice

5781 Van Allen Way
Carlsbad, CA 92008
Phone: 760.603.7200
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Description:

Recombinant human full length protein, inactive, GST-tagged, expressed in *E. coli*.

Specific Activity:

Approximately 0.0% activity as compared to the active form of the kinase.

Concentration:

0.76 mg/mL total protein as measured using the Bradford protein assay with BSA as a standard.

Calculated **11,200 nM**.

Aliases:

p38, Mxi2, SAPK2A

Storage and Handling:

Store at -80°C. At first use, aliquot and store at -80°C to avoid multiple freeze-thaws. If properly stored at -80°C, this product is guaranteed for 6 months from date of purchase.

Storage Buffer:

20 mM Tris (pH 7.5), 150 mM NaCl, 0.5 mM EDTA, 0.01% Triton® X–100, 2 mM DTT and 50% Glycerol.

QUALITY ASSURANCE

Activation Test:

The coupled MAPK14 activation assay uses active MAP2K6, mutant (PV3293) to phosphorylate MAPK14. The activated MAPK14 then phosphorylates myelin basic protein (MBP). The assay was set up in two stages: 1st MAPK14 phosphorylation by MAP2K6, mutant without ^{32}P-ATP; and 2nd myelin basic protein (MBP) phosphorylation by activated MAPK14 in the presence of ^{32}P-ATP. The basal activity of MAPK14 was also assayed in the absence of MAP2K6, mutant.

Dilution Buffer:

50 mM Tris (pH 7.5), 0.01% NP−40, 0.1 mg/mL BSA, 2 mM DTT and 1 mM Na_3VO_4.

Assay Conditions:

Both the active and inactive enzymes were diluted in enzyme dilution buffer and the reaction was set up in two stages. In stage one, MAPK14, inactive (3.33 µg/mL final concentration) was incubated with MAP2K6, mutant (PV3293) (0.83 µg/mL final concentration) or with dilution buffer for basal MAPK14, inactive activity in 50 mM Tris (pH 7.5), 10 mM $MgCl_2$, 1 mM EGTA, 2 mM DTT, 0.02% NP-40, 200 µM ATP for 30 minutes at 30°C. In stage two, 5 µL of the above reaction mix was transferred to 25 µL of reaction mix containing 50 mM Tris (pH 7.5), 10 mM $MgCl_2$, 1 mM EGTA, 2 mM DTT, 0.02% NP-40, 500 µM ATP, 1 mg/mL MBP substrate per reaction and trace [^{32}P]-γ-ATP and incubated for 10 minutes at 30°C.

Gel Information for MAPK14 (p38 alpha), Inactive

Page Description: The SDS-PAGE and/or Native PAGE were run on 4-20% Tris-Glycine Novex™ gels (Catalog #: EC6025BOX).

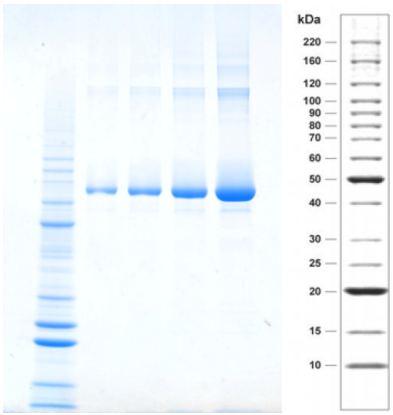
Lane 1: Invitrogen™ BenchMark™ Protein Ladder (Catalog #: 10747-012).

Lane 2: 0.5 µg

Lane 3: 1.0 µg

Lane 4: 2.5 µg

Lane 5: 5.0 µg



Purity:

85% as determined by a SDS-PAGE gel stained with SimplyBlue™ SafeStain.

Molecular Weight:

67.6 kDa. Calculated from the protein sequence(s).

Mass Spectrometry:

MAPK14 (p38 alpha), Inactive was subjected to proteolytic digest followed by mass spec analysis. The resulting MS/MS data verified MAPK14 (p38 alpha) identity by comparison against the amino acid sequence(s) of the recombinant protein.

Protein sequence alignment with reference sequence(s)

GenBank Accession Number: NP_620581

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  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL GST TAG
  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL IVGN MAPK14 (p38 alpha)
  1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- NP_620581

101 DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
101 DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
  1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

201 WPLQGWQATF GGGDHPPKSD LVPRGS
201 WPLQGWQATF GGGDHPPKSD LVPRGSMSQE RPTFYRQELN KTIWEVPERY QNLSPVGSGA YGSVCAAFDT KTGLRVAVKK LSRPFQSIIH AKRTYRELRL
  1 ---------- ---------- ------MSQE RPTFYRQELN KTIWEVPERY QNLSPVGSGA YGSVCAAFDT KTGLRVAVKK LSRPFQSIIH AKRTYRELRL

226
301 LKHMKHENVI GLLDVFTPAR SLEEFNDVYL VTHLMGADLN NIVKCQKLTD DHVQFLIYQI LRGLKYIHSA DIIHRDLKPS NLAVNEDCEL KILDFGLARH
 75 LKHMKHENVI GLLDVFTPAR SLEEFNDVYL VTHLMGADLN NIVKCQKLTD DHVQFLIYQI LRGLKYIHSA DIIHRDLKPS NLAVNEDCEL KILDFGLARH

226
401 TDDEMTGYVA TRWYRAPEIM LNWMHYNQTV DIWSVGCIMA ELLTGRTLFP GTDHIDQLKL ILRLVGTPGA ELLKKISSES ARNYIQSLTQ MPKMNFANVF
175 TDDEMTGYVA TRWYRAPEIM LNWMHYNQTV DIWSVGCIMA ELLTGRTLFP GTDHIDQLKL ILRLVGTPGA ELLKKISSES ARNYIQSLTQ MPKMNFANVF

226
501 IGANPLAVDL LEKMLVLDSD KRITAAQALA HAYFAQYHDP DDEPVADPYD QSFESRDLLI DEWKSLTYDE VISFVPPPLD QEEMES.
275 IGANPLAVDL LEKMLVLDSD KRITAAQALA HAYFAQYHDP DDEPVADPYD QSFESRDLLI DEWKSLTYDE VISFVPPPLD QEEMES
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* highlighted residues denote differences from the reference protein sequence(s).

Chevohn Joseph, Director, Quality Date: 29/Jun/2023