Certificate of Analysis
MAP3K8 (COT), 100 µg

Mitogen-Activated Protein Kinase Kinase Kinase 8, GST-tagged

Part Number: PR7887A
Lot Number: 2578622E
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 protein, catalytic domain (amino acids 30-397), GST-tagged, expressed in insect cells. Activated *in vitro* via auto-phosphorylation.

Specific Activity:

979 nmoles of phosphate transferred to myelin basic protein (MBP) per minute per mg of total protein Activity determined at a final protein concentration of 1.38 µg/mL.

Concentration:

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

Calculated **4,580 nM**.

Aliases:

EST, ESTF

Storage and Handling:

For maximum recovery please spin prior to use. Unless noted below, aliquots of the 5 ug, 10ug and 20ug sizes of kinase are not recommended as materials can be used in original packaging until exhausted. For larger sizes, the number of freeze/thaws may be reduced by preparing aliquots, aliquots below 20 µL are not recommended. **Please never store a kinase diluted.** If properly stored at −80°C, this product is guaranteed for 6 months from date of purchase.

Storage Buffer:

50 mM Tris (pH 7.5), 150 mM NaCl, 0.05 mM EDTA, 0.02% Triton® X–100, 2 mM DTT and 50% Glycerol.

QUALITY ASSURANCE

Dilution Buffer:

20 mM Tris (pH 7.5), 0.02% Triton® X−100, 0.1 mg/mL BSA, 2 mM DTT, 0.5 mM Na_3VO_4 and 10% Glycerol.

Assay Conditions:

MAP3K8 (COT) was assayed in a two stage coupled reaction involving cascade phosphorylations of inactive MAP2K1 Wild–Type (P3093) and inactive MAPK1 (PV3314) followed by phosphorylation of MBP substrate by activated MAPK1. The enzymes were diluted in enzyme dilution buffer before adding to the reaction. In stage one, MAP3K8 (8.33 µg/mL final concentration) is incubated with MAP2K1 (33 µg/mL final concentration) and MAPK3 (33 µg/mL final concentration) in 25 mM HEPES (pH 7.5), 0.01% Triton® X–100, 10 mM $MgCl_2$, 0.5 mM EGTA, 0.5 mM Na_3VO_4, 5 mM β–glycerophosphate, 2.5 mM DTT and 200 µM ATP for 30 minutes at 30°C. In stage two, 5 µL of the above reaction mix is transferred to 25 µL of a reaction mix containing 25 mM HEPES (pH 7.5), 0.01% Triton®X–100, 10 mM $MgCl_2$, 0.5 mM EGTA, 0.5 mM Na_3VO_4, 5 mM β–glycerophosphate, 2.5 mM DTT, 200 µM ATP, 667 µg/mL MBP and trace [^{32}P]–γ–ATP incubated for 10 minutes at 30°C.

Gel Information for MAP3K8 (COT)

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

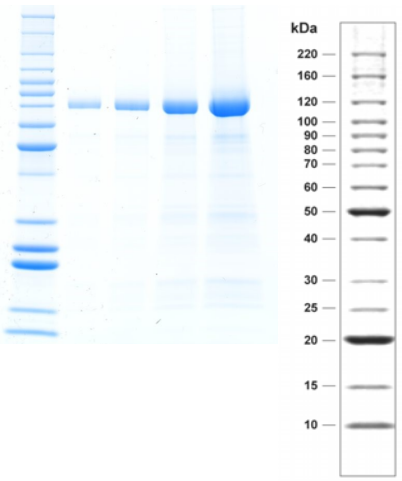
Lane 1: Invitrogen™ BenchMark™ Protein Ladder (Catalog #: 10747-012).

Lane 2: 0.5 µg MAP3K8 (COT)

Lane 3: 1.0 µg MAP3K8 (COT)

Lane 4: 2.5 µg MAP3K8 (COT)

Lane 5: 5.0 µg MAP3K8 (COT)



Purity:

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

Molecular Weight:

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

Mass Spectrometry:

MAP3K8 (COT) was subjected to proteolytic digest followed by mass spec analysis. The resulting MS/MS data verified MAP3K8 (COT) identity by comparison against the amino acid sequence(s) of the recombinant protein.

Protein sequence alignment with reference sequence(s)

GenBank Accession Number: NP_005195

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  1 MAPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL GST TAG
  1 MAPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL IVGN MAP3K8 (COT)
 31 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- NP_005195

101 DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
101 DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
 31 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

201 WPLQGWQATF GGGDHPPKSD LVPR
201 WPLQGWQATF GGGDHPPKSD LVPRHNQTSL YKKAGFEGDR TMENLYASEE PAVYEPSLMT MCQDSNQNDE RSKSLLLSGQ EVPWLSSVRY GTVEDLLAFA
 31 ---------- ---------- ---------- ---------- -MENLYASEE PAVYEPSLMT MCQDSNQNDE RSKSLLLSGQ EVPWLSSVRY GTVEDLLAFA

224
301 NHISNTAKHF YGQRPQESGI LLNMVITPQN GRYQIDSDVL LIPWKLTYRN IGSDFIPRGA FGKVYLAQDI KTKKRMACKL IPVDQFKPSD VEIQACFRHE
 89 NHISNTAKHF YGQRPQESGI LLNMVITPQN GRYQIDSDVL LIPWKLTYRN IGSDFIPRGA FGKVYLAQDI KTKKRMACKL IPVDQFKPSD VEIQACFRHE

224
401 NIAELYGAVL WGETVHLFME AGEGGSVLEK LESCGPMREF EIIWVTKHVL KGLDFLHSKK VIHHDIKPSN IVFMSTKAVL VDFGLSVQMT EDVYFPKDLR
189 NIAELYGAVL WGETVHLFME AGEGGSVLEK LESCGPMREF EIIWVTKHVL KGLDFLHSKK VIHHDIKPSN IVFMSTKAVL VDFGLSVQMT EDVYFPKDLR

224
501 GTEIYMSPEV ILCRGHSTKA DIYSLGATLI HMQTGTPPWV KRYPRSAYPS YLYIIHKQAP PLEDIADDCS PGMRELIEAS LERNPNHRPR AADLLKHEAL
289 GTEIYMSPEV ILCRGHSTKA DIYSLGATLI HMQTGTPPWV KRYPRSAYPS YLYIIHKQAP PLEDIADDCS PGMRELIEAS LERNPNHRPR AADLLKHEAL

224
601 NPPREDQPR.
389 NPPREDQPR
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* highlighted residues denote differences from the reference protein sequence(s).

Chevohn Joseph, Director, Quality Date: 16/Feb/2023