

Certificate of Analysis

MAP3K8 (COT), 100 µg

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

ThermoFisher
SCIENTIFIC

Part Number: PR7887A

Lot Number: 2578622D

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 µg, 10 µg and 20 µg 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 Na₃VO₄ 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 MAPK1 (33 µg/mL final concentration) in 25 mM HEPES (pH 7.5), 0.01% Triton® X-100, 10 mM MgCl₂, 0.5 mM EGTA, 0.5 mM Na₃VO₄, 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 MgCl₂, 0.5 mM EGTA, 0.5 mM Na₃VO₄, 5 mM β-glycerophosphate, 2.5 mM DTT, 200 µM ATP, 667 µg/mL MBP and trace [³²P]-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).

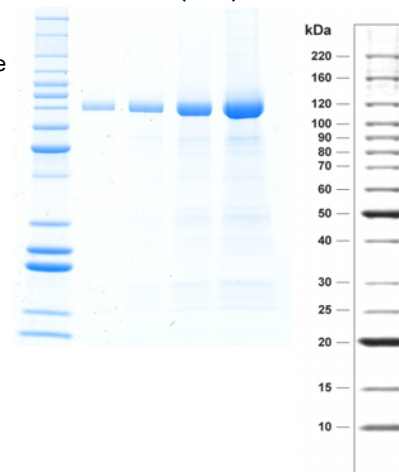
Lane 1: Invitrogen™ BenchMark™ Protein Ladder (Catalog #: 10747-012).

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

1	MAPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	GDVKLTQDMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	GST TAG
1	MAPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	GDVKLTQDMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	IVGN MAP3K8 (COT)
31											NP_005195
101	DIRYGVSRIA	YSKDFETLKV	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	KRIEAI PQID	KYLKSSSKYIA	
101	DIRYGVSRIA	YSKDFETLKV	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	KRIEAI PQID	KYLKSSSKYIA	
31											
201	WPLQGWQATF	GGGDHPPKSD	LVPR								
201	WPLQGWQATF	GGGDHPPKSD	LVPRHNTSL	YKKAGFEGDR	TMENLYASEE	PAVYEPSLMT	MCQDSNQND E	RSKSLLLSGQ	EVPLWSSVRY	GTVEDLLAFA	
31					-MENLYASEE	PAVYEPSLMT	MCQDSNQND E	RSKSLLLSGQ	EVPLWSSVRY	GTVEDLLAFA	
224											
301	NHISNTAKHF	YQRPQESGI	LLNMVITPON	GRYQIDSDVL	LIPWKLT YRN	IGSDFIPRGA	FGKVYLAQDI	KTKKRMACKL	IPVDQFKPSD	VEIQACFRHE	
89	NHISNTAKHF	YQRPQESGI	LLNMVITPON	GRYQIDSDVL	LIPWKLT YRN	IGSDFIPRGA	FGKVYLAQDI	KTKKRMACKL	IPVDQFKPSD	VEIQACFRHE	
224											
401	NIAELYGAVL	WGETVHLFME	AGEGGSVLEK	LESCGPMREF	EIIWVTKHVL	KGLDFLH SKK	VIHHDIKPSN	IVFMSTKAVL	VDFGLSVQMT	EDVYFPKDLR	
189	NIAELYGAVL	WGETVHLFME	AGEGGSVLEK	LESCGPMREF	EIIWVTKHVL	KGLDFLH SKK	VIHHDIKPSN	IVFMSTKAVL	VDFGLSVQMT	EDVYFPKDLR	
224											
501	GTEIYMSPEV	ILCRGHSTKA	DIYSLGATLI	HMQTGTPPWV	KRYPRSA YPS	YLYIIHKQAP	PLEDIADDCS	PGMRELIEAS	LERNPNHRPR	AADLLKHEAL	
289	GTEIYMSPEV	ILCRGHSTKA	DIYSLGATLI	HMQTGTPPWV	KRYPRSA YPS	YLYIIHKQAP	PLEDIADDCS	PGMRELIEAS	LERNPNHRPR	AADLLKHEAL	
224											
601	NPPREDQPR.										
389	NPPREDQPR										

* highlighted residues denote differences from the reference protein sequence(s).



Chevoyn Joseph, Director, Quality

Date: 16/Feb/2023

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