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Certificate of Analysis

MOK, active
(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-960, 14-960-K, 14-960M

Parent Lot # D15DP013N

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 and *C*-terminal 6His tagged, recombinant, human MOK amino acids 1-343 expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography followed by glutathione agarose.
Purity 88% by SDS-PAGE and Coomassie blue staining. MW = 70kDa.

Specific Activity (Parent lot# D15DP013N): 410U/mg, where one unit of MOK activity is defined as 1nmol phosphate incorporated into 300µM RSRSRSRSRSRSRSR per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.17mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

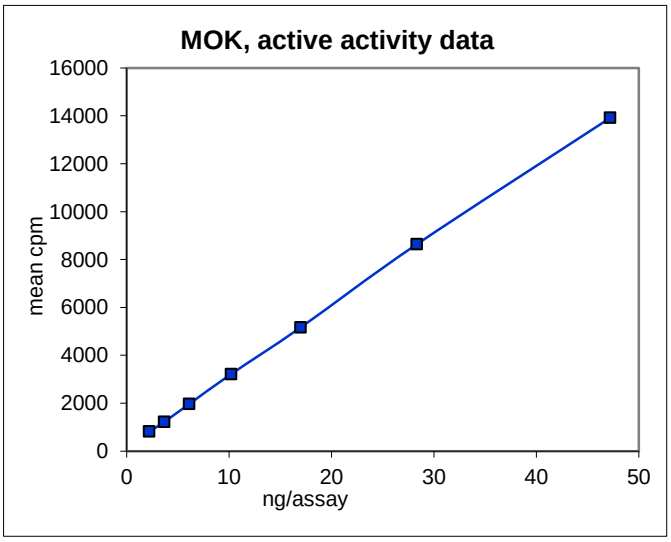
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C..

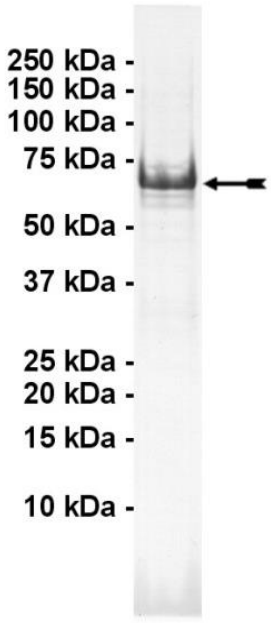
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 2–47ng of this lot of enzyme phosphorylated 300µM RSRSRSRSRSRSRSR 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 identity as MOK 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 MOK, active

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **RSRSRSRSRSRSRSR**: Use at a final assay concentration of 300µM. Prepare a 3mM stock and add 2.5µl of stock per assay point.
3. **MOK, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2–47ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x MgAc/[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 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **RSRSRSRSRSRSRSR**.
3. Add **2.5µl (2–47ng) MOK, 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 75mM 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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MOK, active Sequence Information

Protein	human MOK
Tags	*N*-terminal GST and *C*-terminal 6His
Native sequence	M230 of the recombinant protein is equivalent to M1 of human MOK
Accession number	GenBank NM_014226.2

Recombinant MOK 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 LVPRGSKEFM KNYKAIGKIG
241 EGTFSEVMKM QSLRDGNYYA CKQMKQRFES IEQVNNLREI QALRRLNPHP NILMLHEVVF
301 DRKSGSLALI CELMDMNIYE LIRGRRYPLS EKKIMHYMYQ LCKSLDHIHR NGIFHRDVKP
361 ENILIKQDVL KLGDFGSCRS VYSKQPYTEY ISTRWYRAPE CLLTDGFYTY KMDLWSAGCV
421 FYEIASLQPL FPGVNELDQI SKIHDVIGTP AQKILTKFKQ SRAMNFDFPF KKGSGIPLLT
481 TNLSPQCLSL LHAMVAYDPD ERIAAHQALQ HPYFQEQRKT EKRALGSHRK AGFPEHPVAP
541 EPLSNSCQIS KEGRKQKQSL KQEEDRPKRR GPENLYFQGV EACQLGTDDY DIPTTHHHHH
601 H
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Recombinant MOK 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
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1801 cattaa
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Reviewed and approved by site quality representative.