

## 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 RSRSRSRSRSRSR 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.

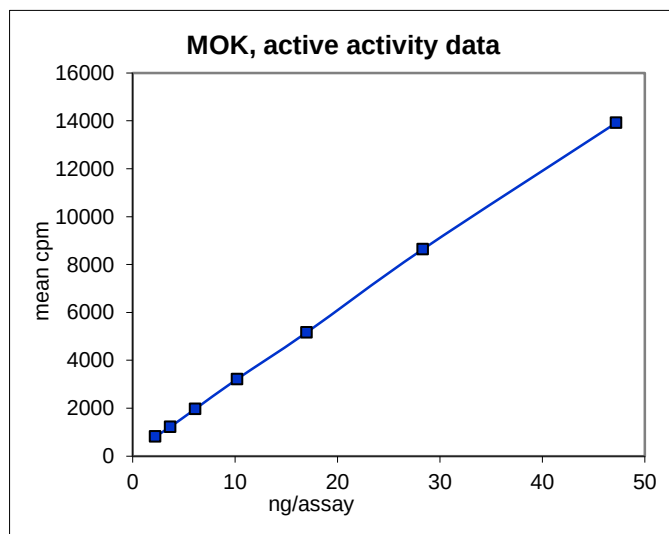
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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..

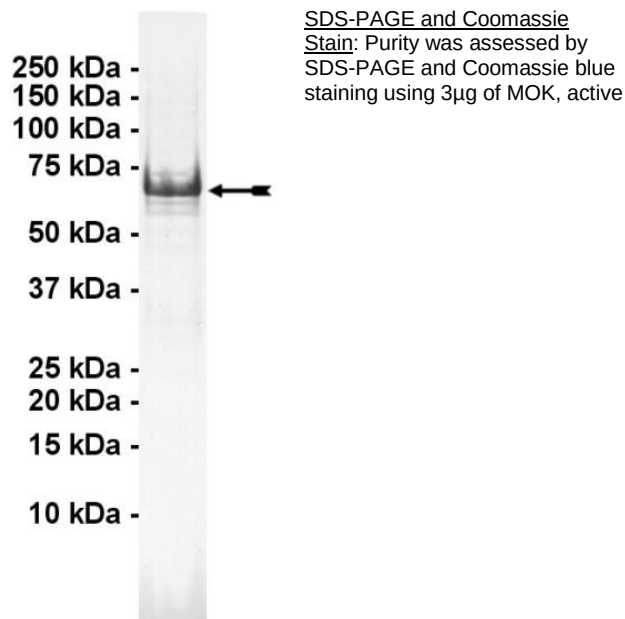
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 2–47ng of this lot of enzyme phosphorylated 300µM RSRSRSRSRSRSR 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



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### Kinase Assay Protocol

#### Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **RSRSRSRSRSRSR:** Use at a final assay concentration of 300 $\mu$ M. Prepare a 3mM stock and add 2.5 $\mu$ l 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. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x MgAc/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

#### Assay Procedure (96 well plate format):

1. Add 5 $\mu$ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 $\mu$ l of **RSRSRSRSRSRSR**.
3. Add **2.5 $\mu$ l (2–47ng) MOK, active**.
4. Add 5 $\mu$ l of dH<sub>2</sub>O.
5. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 $\mu$ l of 3% phosphoric acid.
8. Transfer a 10 $\mu$ l 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 1 $\mu$ l of 30% phosphoric acid.

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### MOK, active Sequence Information

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

### Recombinant MOK amino acid sequence:

```

1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSM  IIRYIADKH  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLPEML  KMFEDRLCH  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPOID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSKEFM  KNYKAIGKIG
241  EGTTFSEVMKM  QSLRDGNYYA  CKQMKQRFES  IEQVNNLREI  QALRRLNPHP  NILMLHEVVF
301  DRKSGSLALI  CELMDMNIYE  LIRGRRYPLS  EKKIMHYMYQ  LCKSLDHIHR  NGIFHRDVKP
361  ENILIKQDVL  KLGDFGSCRS  VYSKQPYTEY  ISTRWYRAPE  CLLTDGFYTY  KMDLWSAGCV
421  FYEIASLQPL  FPGVNELDQI  SKIHDVIGTP  AQKILTKFKQ  SRAMNFDFFP  KKGSGIPLLT
481  TNLSPQCLSL  LHAMVAYDPD  ERIA AHQALQ  HPYFQEQRKT  EKRALGSHRK  AGFPEHPVAP
541  EPLSNQCQIS  KEGRKQKQSL  KQEEDRPKRR  GPENLYFQGV  EACQLGTDDY  DIPTTHHHHH
601  H

```

### Recombinant MOK nucleotide sequence:

```

1  atgtccccta  tactagggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggttg  gagtttccca  atcttcctta  ttatattgat
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatacggt  atatagctga  caagcacaac
241  atgttggttg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
301  gatattagat  acggtgtttc  gagaattgca  tatagtaaag  actttgaaac  tctcaaagtt
361  gattttctta  gcaagctacc  tgaaatgctg  aaaatggtcg  aagatcgttt  atgtcataaa
421  acatatTTaa  atggtgatca  tgtaaccat  cctgacttca  tggtgtatga  cgctcttgat
481  gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601  tggcctttgc  agggctggca  agccactgtt  ggtggtggcg  accatcctcc  aaaaatcgga
661  ctgggttccg  gtggatccaa  ggaactcatg  aagaactata  aagcaattgg  aaaaatagga
721  gagggaaact  tttctgaagt  tatgaagatg  caaagcctga  gagatggaaa  ctactatgca
781  tgtaaacaaa  tgaagcagcg  ctttgaaagt  attgagcaag  tcaacaacct  acgagagatc
841  caagcactga  ggcgcctgaa  tccgcaccca  aacattctta  tggtgcatga  agtgggtttt
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961  ctaatacgag  ggagaagata  cccattatca  gaaaaaaaaa  ttatgacta  tatgtaccag
1021  ttatgtaagt  ccctggatca  tattcacaga  aatggaatat  ttcacagaga  tgtaaaacca
1081  gaaaatatac  taataaagca  ggatgtcctg  aaattagggg  actttggctc  ctgccggagt
1141  gtctattcca  agcagccgta  cacggaatac  atctccacc  gctgggtacc  ggccccggag
1201  tgtctcctca  ctgatgggtt  ctacacgtac  aagatggacc  tgtggagcgc  cggctgtgtg
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1381  tcgagagcta  tgaattttga  ttttcctttt  aaaaagggat  caggaatacc  tctactaaca
1441  accaatttgt  ccccaaatg  cctctccctc  ctgcacgcaa  tgggtggccta  tgatcccgat
1501  gagagaatcg  ccgcccacca  ggccctgcag  caccctact  tccaagaaca  gaggaaaaca
1561  gagaagcggg  ctctgggcag  ccacagaaaa  gctggctttc  cgagacccc  tgtggcaccg
1621  gaaccactca  gtaacagctg  ccagatttcc  aaggagggca  gaaagcagaa  acagtcctta
1681  aagcaagagg  aggaccgtcc  caagagacga  ggaccggaaa  acctgtattt  tcagggcgtg

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## Certificate of Analysis

```
1741 gaagcctgtc aattgggcac ggacgattac gacatcccga ctaccacca tcaccatcac  
1801 catta
```

Reviewed and approved by site quality representative.

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