

Certificate of Analysis

MSSK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-665, 14-665-K, 14-665M

Parent Lot # 1606078

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 6His-tagged, recombinant, human, full length MSSK1, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 67% by SDS-PAGE and Coomassie blue staining. MW = 65.7kDa.

Specific Activity (Parent lot# 1606078): 116U/mg, where one unit of MSSK1 activity is defined as 1nmol phosphate incorporated into 50μM PKCtide (ERMPRKRQGSVRRRV) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.484mg/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.

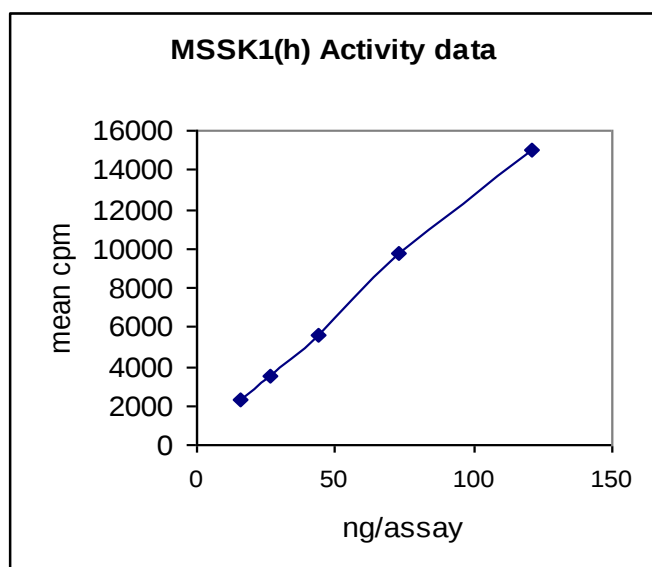
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

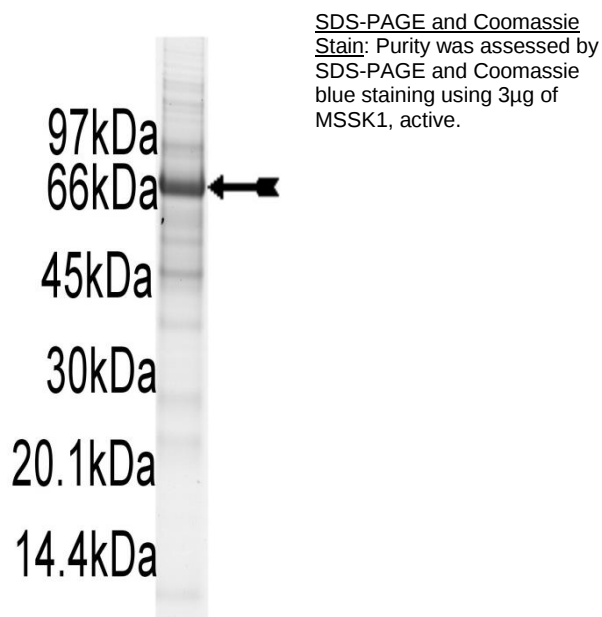
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 16–121ng of this lot of enzyme phosphorylated 50μM PKCtide 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 product identity as MSSK1 with the translated native sequence listed on page three.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **PKCtide:** Use at a final assay concentration of 50 μ M. Make up a 500 μ M stock. Add 2.5 μ l of stock per assay point
3. **MSSK1, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 16–121ng per assay point.
4. **$[\gamma\text{-}^{33}\text{P}]\text{ATP}$:** 2.5 x magnesium acetate/ $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ cocktail: 25mM MgAc and 0.25mM ATP to which is added $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **PKCtide (ERM RPRKRQGSVRRRV)**.
3. Add **2.5 μ l (16–121ng) MSSK1, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ 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 μ l of 30% phosphoric acid.

Certificate of Analysis

MSSK1 Sequence Information

<u>Protein</u>	Human MSSK1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of the recombinant protein is equivalent to M1 of human MSSK1
<u>Accession number</u>	GenBank NM_014370. The recombinant protein contains an insertion of 33 amino acid residues after G348 of the amino acid sequence shown below (equivalent to G318 of the native protein sequence). This conflict is reported in GenBank BX282601 and CD106669.

Recombinant MSSK1 amino acid sequence:

```

1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MSASTGGGGD SGGSGGSSSS SQASCGPESS
61 GSELALATPV PQMLQGLLGS DDEEQEDPKD YCKGGYHPVK IGDVFNGRYH VVRKLGWGHF
121 STVWLCWDIQ RKRFVALKVV KSAGHYTETA VDEIKLLKCV RSDPSDPKR ETIVQLIDDF
181 RISGVNGVHV CMVLEVLGHQ LLKWIISNY QGLPVPCVKS IVRQVLHGLD YLHTKCKIIH
241 TDIKPENILL CVGDAYIRRL AAEATEWQQA GAPPPSRIV STAPQEVLTG KLSKNKRKKM
301 RRKRKQKRL LEERLRLQR LEAMEAATQA EDSGLRLDGG SGSTSSSGCH PGGARAGPSP
361 ASSSPAPGGG RSLSAGSQTG GFSGSLFSPA SCSILSGSSN QRETGGLLSP STPFGASNLL
421 VNPLEPQNAD KIKIKIADLG NACWVHKHFT EDIQRQYRA VEVLIGAEYG PPADIWSTAC
481 MAFELATGDY LFEPHSGEDY SRDEDHIAHI VELLGDIPPA FALSGRYSRE FFNRRGELRH
541 IHNLKHGWL YVLMKEYEWP LEQATQFSAF LLPMEYIPE KRASAADCLQ HPWLNP

```

Recombinant MSSK1 nucleotide sequence:

```

1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc atgagcgcca gcacgggcgg tgggtggggc
121 agcggcggca gcggcggcag tagcagcagc tcacaggcct cctgcgggcc cgagtcctcg
181 ggctccgaac tagccctggc cacaccggtg cctcagatgc tgcagggcct tctgggctcc
241 gacgacgagg aacaggaaga ccccaaagac tactgcaagg gcggctacca ccctgtgaag
301 atcgcgacg tgttcaatgg gcggtaccac gtggtgcgca aactgggctg gggccacttc
361 tccaccgtct ggctctgctg ggacatccag cgcaagcgct ttgtggccct caaagtgggtg
421 aagagtgcgg ggcattacac ggagacagct gtggatgaga tcaagctcct gaaatgtgtc
481 cgggacagcg accccagtga ccccaaaaga gagaccattg tccagctcat tgatgacttc
541 aggatctcag gagtcaatgg agtccatgtg tgcattggtg tggaggtgct gggccaccag
601 ctctctcaat ggatcatcaa gtccaactac cagggcctgc cgtgcccctg cgtgaagagc
661 atcgtgaggc aggtgctgca cggcctggac tacctccaca ccaagtgcaa gatcatccac
721 acggacatca agcccagaaa catcttgctg tgtgtggggg acgcttacat caggcgcctg
781 gctgccgagg ccacggagtg gcaacaggca ggggcgccgc cccctccccg ctccatagtc
841 agcactgccc cccaggaggt cttgaccggt aagctgtcca aaaacaagag gaagaagatg
901 aggcgcaaac ggaacagca gaagcggtg ctggaggagc ggctgcggga cctgcagagg
961 ctggaggcca tggaggctgc caccaggctg gaggactctg gcttgagact agacgggggc
1021 agcggctcca catctcttc aggtgtcac cccggggggc ccagagcagg tccctcccca
1081 gcctcttcct ccccgcccc ggggggcggc cgtagcctca gcgcgggctc acagacctca
1141 ggcttctccg gctccctctt ctctctgcc tcctgtctca tcctctccgg ctctgccaat
1201 cagcgagaga ccgggggcct cctgtgcct agcacacat tcggtgcctc gaacctcctg
1261 gtgaaccccc tggagcccca aaatgcagat aagatcaaga tcaagatcgc agacctgggc
1321 aacgcctgct gggtgcaaaa gcacttcacg gaagacatcc agactcggca gtaccggggc
1381 gtcgaggctg tgatcgcgcg cgaatacggc ccccgggcag acatctggag cacagcctgc
1441 atggccttcg agctggccac tgggtactac ctgttcgagc cgattcttgg agaagactac
1501 agtcgtgatg aggaccacat cgctcacata gtggagcttc tgggggacat ccccccagcc
1561 ttcgccctct caggccgcta ttccgggag ttcttcaacc ggagaggaga gctgcggcac

```

Certificate of Analysis

```
1621 atccacaatc tcaagcactg gggcctgtac gaggtactca tggaaaagta cgagtggccc
1681 ctagagcagg ccacacagtt cagcgccttt ctgctgcca tgatggagta catccccgaa
1741 aagcgggcca gtgccgctga ctgcctccag caccctggc tcaacccta g
```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 **Eurofins Pharma Discovery Services UK Limited** is an independent member of Eurofins Discovery Services