

Certificate of Analysis

MARK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-651, 14-651-K, 14-651M

Parent Lot # D8MN035U

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 MARK1, amino acids 2-end, expressed by baculovirus in Sf21 cells. Purified using Ni²⁺/NTA agarose. Purity 56.9% by SDS-PAGE and Coomassie blue staining. MW = 92.7kDa.

Specific Activity (Parent lot# D8MN035U): 1304U/mg, where one unit of MARK1, active activity is defined as 1nmol phosphate incorporated into 100µM CHKtide (KKKVSRSGLYRSPSPENLNRRP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.788mg/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.

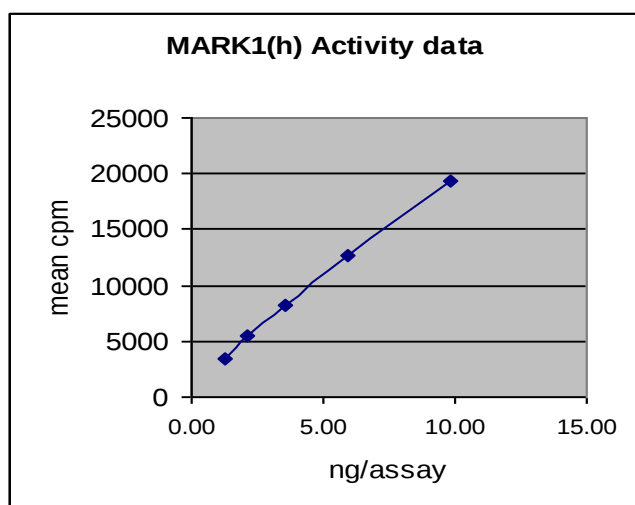
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

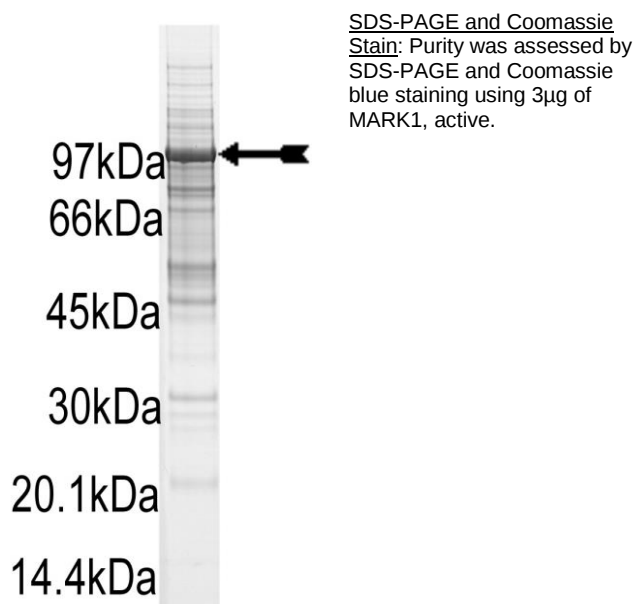
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.3–9.8ng of this lot of enzyme phosphorylated 100µM CHKtide (KKKVSRSGLYRSPSPENLNRRP) 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 MARK1 with the translated native 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. **100µM CHKtide (KKKVSRSGLYRSPSPENLNRPR):** Use at a final assay concentration of 100µM. Make a 1mM stock. Add 2.5µl of stock per assay point.
3. **MARK1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.3–9.8ng per assay point.
4. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]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 **CHKtide (KKKVSRSGLYRSPSPENLNRPR)**.
3. Add **2.5µl (1.3–9.8ng) MARK1, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ-³³P]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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MARK1 Sequence Information

Protein	Human MARK1
Tags	N-terminal 6His
Native sequence	S31 of the recombinant protein is equivalent to S2 of human MARK1
Accession number	Genbank AF154845. The recombinant protein contains the amino acid substitutions V16E and T20S with reference to AF154845. These mutations are reported in GenBank NM_018650, AK098103 and BC068608.

Recombinant MARK1 amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF SARTPLPTVN ERDTENHTSV DGYTEPHIQP
61 TKSSSRQNIP RCRNSITSAT DEQPHIGNYR LQKTIGKGNF AKVKLARHVL TGREVAVKII
121 DKTQLNPTSL QKLFREVRIM KILNHPNIVK LFEVIETEK LYLVMYASG GEVFDYLVAH
181 GRMKEKEARA KFRQIVSAVQ YCHQKYIVHR DLKAENLLLD GDMNIKIADF GFSNEFTVGN
241 KLDTCGSP YAAPELFQ GK KYDGPEDVW SLGVILYTLV SGSLPFDGQN LKELRERVLR
301 GKYRIPFYS TDCENLLKKL LVLNPIKRG S LEQIMKDRWM NVGHEEEELK PYTEPDPDFN
361 DTKRIDIMVT MGFARDEIND ALINQKYDEV MATYILLGRK PPEFEGGESL SSGNLCQRSR
421 PSSDLNNSTL QSPAHLKVQR S ISANQKQRR FSDHAGPSIP PAVSYTKRPQ ANSVESEQKE
481 EWDKDVARKL GSTTVGSKSE MTASPLVGPE RKKSSSTIPSN NVYSGGSMAR RNTYVCERTT
541 DRYVALQNGK DSSLTEMSVS SISSAGSSVA SAVPSARPRH QKSMSTSGHP IKVTLPTIKD
601 GSEAYRPGTT QRVPAASPSA HSISTATPDR TRFPRGSSSR STFHGEQLRE RRSVAYNGPP
661 ASPSHETGAF AHARRGTSTG IISKITSKFV RRDPSERGEAS GRDTSTRSTS GEPKERDKEE
721 GKDSKPRSLR FTWSMKTTSS MDPNDMMREI RKVLDANNCD YEQKERFLLF CVHGDARQDS
781 LVQWEMEVC LPRLSLNGVR FKRISGTSIA FKNIAASKIAN ELKL

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Recombinant MARK1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc tcggcccggg cgccattgcc gacggtgaac
121 gagcgggaca cggaaaaatca tacatctgtg gatggatata ctgaaccaca catccagcct
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541 ggaagaatga aagagaaaga ggcccgtgca aaatttaggc agattgtatc tgcgttacag
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721 aaattggaca cattttgtgg aagcccaccc tatgctgctc ccgagctttt ccaaggaaag
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1201 ccacctgaat ttgaaggtgg tgaatcgta tccagtggaa acttgtgtca gaggtcccgg
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1321 agtatctcag caaatcagaa gcagcggcgt ttcagtgtat atgctggtcc atccattcct
1381 cctgctgtat catataccaa aagacctcag gctaacagtg tggaaagtga acagaaagag

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1441 gagtgggaca aagatgtggc tcgaaaactt ggcagcacia cagttggatc aaaaagcgag
1501 atgactgcaa gccctcttgt agggccagag aggaaaaaat cttcaactat tccaagtaac
1561 aatgtgtatt ctggaggtag catggcaaga aggaatacat atgtctgtga aaggaccaca
1621 gatcgatacg tagcattgca gaatggaaaa gacagcagcc ttacggagat gtctgtgagt
1681 agcatactct ctgcaggctc ttctgtggcc tctgtgtcc cctcagcacg acccgccac
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2161 ggtaaagatt ctaagccgct ttctttgcgg ttcacatgga gtatgaagac cactagttca
2221 atggacccta atgacatgat gagagaaatc cgaaaagtgt tagatgcaa taactgtgat
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2461 gagcttaagc tgtaa

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Reviewed and approved by site quality representative.

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