

Certificate of Analysis

Mnk2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-664, 14-664-K, 14-664M

Parent Lot # WAE0126

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 Mnk2, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 63% by SDS-PAGE and Coomassie blue staining. MW = 50.5kDa.

Specific Activity (Parent lot# WAE0126): 87U/mg, where one unit of Mnk2, active activity is defined as 1nmol phosphate incorporated into 0.333mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.48mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamide, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

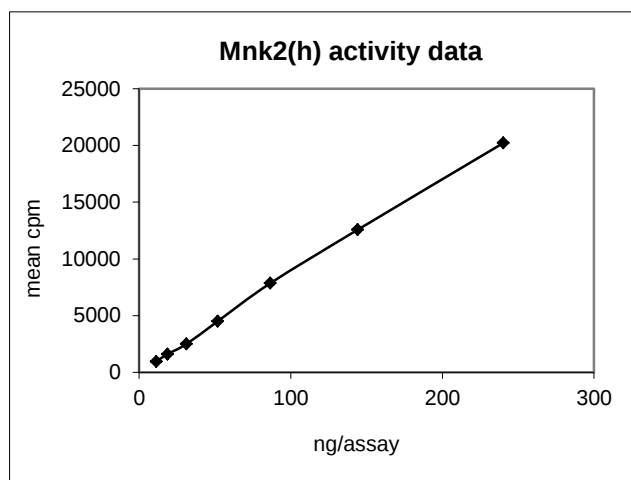
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

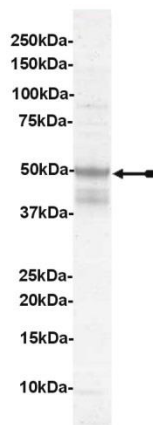
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 11.20–240ng of this lot of enzyme phosphorylated 0.333mg/ml myelin basic protein (MBP) 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 Mnk2 with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Mnk2, active.

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
- 2. Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.333mg/ml. Make a 3.33mg/ml stock. Add 2.5µl of stock per assay point.
- 3. Mnk2, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 11.20–240ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 2 5mM 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 **myelin basic protein (MBP)**.
3. Add **2.5µl (11.20–240ng) Mnk2, 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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Mnk2 Sequence Information

Protein	Human Mnk2
Tags	N-terminal 6His
Native sequence	M31 of the recombinant protein is equivalent to M1 of human Mnk2
Accession number	GenBank NM_017572

Recombinant Mnk2 amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF MVQKKPAELQ GFHRSFKGQN PFELAFSLDQ
61 PDHGDSDFGL QCSARPDMPA SQPIDIPDAK KRGKKKKRGR ATDSFSGRFE DVYQLQEDVL
121 GEGAHARVQT CINLITSQEY AVKIIEKQPG HIRSRVFREV EMLYQCQGHR NVLELIEFFE
181 EEDRFYLVFE KMRGGSILSH IHKRRHFNEL EASVVVQDVA SALDFLHNKG IAHRDLKPEN
241 ILCEHPNQVS PVKICDFDLG SGIKLNGDCS PISTPELLTP CGSAEYMAPE VVEAFSEEAS
301 IYDKRCDLWS LGVILYILLS GYPFVGRGCG SDCGWDRGEA CPACQNMLFE SIQEGKYEFP
361 DKDWAHISCA AKDLISKLLV RDAKQRLSAA QVLQHPWVQG CAPENTLPTP MVLQRWDSHF
421 LLPPHPCRHH VRPGLVVRTV TVNE

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Recombinant Mnk2 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc atggtgcaga agaaaccagc cgaacttcag
121 ggtttccacc gttcgttcaa ggggcagaac ccttcgagc tggccttctc cctagaccag
181 cccgaccacg gagactctga ctttggcctg cagtgtctag cccgtcctga catgcccgcc
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301 gccaccgaca gcttctcggg caggtttgaa gacgtctacc agctgcagga agatgtgtctg
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1321 actgtgaatg agtga

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