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Certificate of Analysis

MINK, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-615, 14-615-K, 14-615M
Parent Lot # 1689136

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 MINK amino acids 1–310, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 86.7% by SDS-PAGE and Coomassie blue staining. MW = 38.7kDa.

Specific Activity (Parent lot# 1689136): 594U/mg, where one unit of MINK, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.107mg/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.

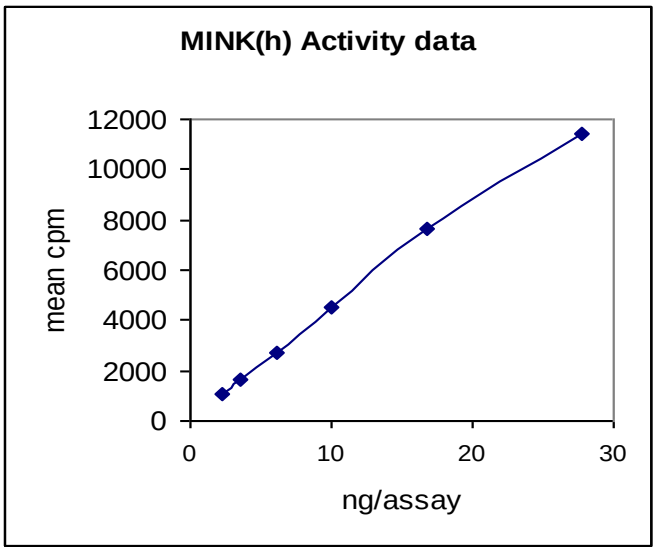
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

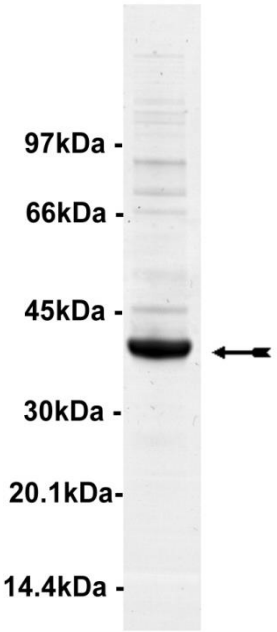
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 2.2–27.7ng of this lot of enzyme phosphorylated 0.33mg/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 identity as MINK 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 MINK, 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 0.33mg/ml. Make up a 3.33mg/ml stock. Use 2.5µl of stock solution per assay point.
3. **MINK, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 2.2–27.7ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[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 **Myelin Basic Protein (MBP)**.
3. Add **2.5µl (2.2–27.7ng) MINK, 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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MINK Sequence Information

Protein human MINK

Tags *N*-terminal 6His

Native sequence M29 of the fusion protein is equivalent to M1 of human MINK

Accession number GenBank NM_015716

Recombinant MINK amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMGSMG DPAPARSLDD IDLSALRDPA GIFELVEVVG
 61 NGTYGQVYKG RHVKTGQLAA IKVMDVTEDE EEEIKQEINM LKKYSHHRNI ATYYGAFIKK
121 SPPGNDDQLW LVMEFCGAGS VTDLVKNTKG NALKEDCIAY ICREILRGLA HLHAHKVIHR
181 DIKGQNVLLT ENAEVKLVDF GVSAQLDRTV GRRNTFIGTP YWMAPEVIAC DENPDATYDY
241 RSDIWSLGIT AIEMAEGAPP LCDMHPMRAL FLIPRNPPPR LKSKKWSKKF IDFIDTCLIK
301 TYLSRPPTEQ LLKFPFIRDQ PTERQVRIQL KDHIDRSR
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Recombinant MINK nucleotide sequence:

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  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatggg atccatgggc gacccagccc ccgcccgcag cctggacgac
121 atcgacctgt ccgccctgcg ggaccctgct gggatctttg agcttgtgga ggtggtcggc
181 aatggaacct acggacaggt gtacaagggt cggcatgtca agacggggca gctggctgcc
241 atcaaggtca tggatgtcac ggaggacgag gaggaagaga tcaaacagga gatcaacatg
301 ctgaaaaagt actctcacca ccgcaacatc gccacctact acggagcctt catcaagaag
361 agccccccgg gaaacgatga ccagctctgg ctggtgatgg agttctgtgg tgctggttca
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481 atctgcaggg agatcctcag gggtctggcc catctccatg cccacaaggt gatccatcga
541 gacatcaagg ggcagaatgt gctgctgaca gagaatgctg aggtcaagct agtggatttt
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961 cccacggagc ggcaggtccg catccagctt aaggaccaca ttgaccgatc ccggtag
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Reviewed and approved by site quality representative.