

Certificate of Analysis

MRCK β , active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-643, 14-643-K, 14-643M

Parent Lot # D8NN076U

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 MRCK amino acids 1–473, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺-NTA agarose. Purity 71.4% by SDS-PAGE and Coomassie blue staining. MW = 55.5kDa.

Formulation: 1.617mg/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.

Specific Activity (Parent lot# D8NN076U): 222U/mg, where one unit of MRCK activity is defined as 1nmol phosphate incorporated into 100 μ M (KKRNRTLTV) per minute at 30°C with a final ATP concentration of 100 μ M.

Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months 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 microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

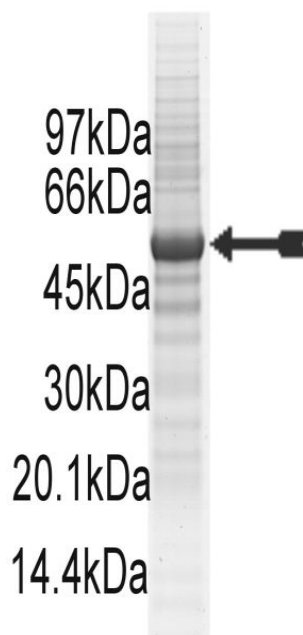
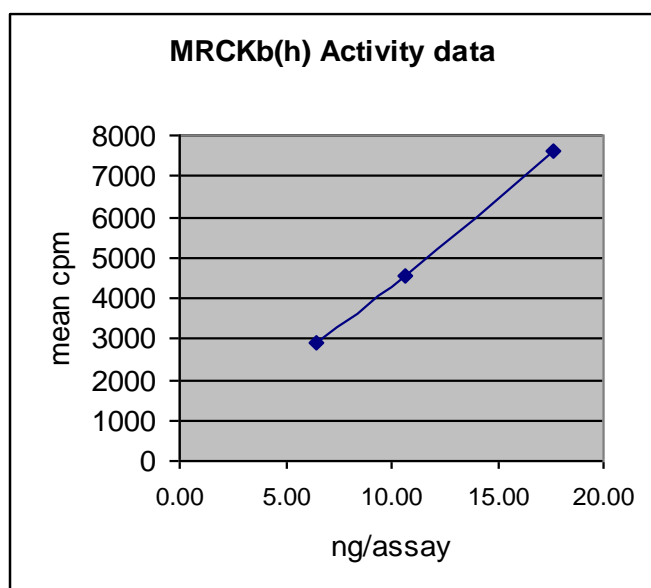
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 6–18ng of this lot of enzyme phosphorylated 100 μ M (KKRNRTLTV) 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 MRCK β 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 3 μ g of MRCK β , active.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. Substrate peptide (KKRNRTLTV):** Use at a final assay concentration of 100µM. Make up a 1mM stock. Add 2.5 µl of stock per assay point.
- 3. MRCKβ, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 6–18ng 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 **Substrate peptide (KKRNRTLTV)**.
3. Add **2.5µl (6–18ng) MRCKβ 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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MRCK β Sequence Information

<u>Protein</u>	human MRCK β
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M1 of recombinant sequence is equivalent to M1 of native human MRCK β
<u>Accession number</u>	GenBank NM_006035

Recombinant MRCK β amino acid sequence:

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1 MSAKVRLLKKL EQLLLDGPWR NESALSVETL LDVVLVCLYTE CSHSALRRDK YVAEFLEWAK
61 PFTQLVKEMQ LHREDFEIIK VIGRGAFGEV AVVKMKNTER IYAMKILNKW EMLKRAETAC
121 FREERDVLVN GDCQWITALH YAFQDENHLY LVMDYYVGGD LLTLLSKFED KLPEDMARFY
181 IGEMVLAIIDS IHQLHYVHRD IKPDNVLLDV NGHIRLADFG SCLKMNDGDT VQSSVAVGTP
241 DYISPEILQA MEDGMGKYGP ECDWWSLGV C MYEMLYGETP FYAESLVETY GKIMNHEERF
301 QFPSHVTDVS EEAKDLIQRL ICSRERRLGQ NGIEDFKKHA FFEGLNWENI RNLEAPYIPD
361 VSSPSDTSNF DVDDDLVRLNT EILPPGSHTG FSGLHLPFIG FTFTTESCF S DRGSLKSIMQ
421 SNTLTKDEDV QRDLEHSLQM EAYERRIRRL EQEKLELSRK LQESTQTVQS LHGHHHHHH

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Recombinant MRCK β nucleotide sequence:

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1 atgtcggcca aggtgcggt caagaagctg gagcagctgc tcctggacgg gccctggcgc
61 aacgagagcg ccctgagcgt ggaacgctg ctcgacgtgc tcgtctgcct gtacaccgag
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