

Certificate of Analysis

MRCK α , active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-691, 14-691-K, 14-691M

Parent Lot # 1606088

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: C-terminal 6His-tagged, recombinant, human MRCK α , amino acids 1–473, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 91.7% by SDS-PAGE and Coomassie blue staining. MW = 55.3kDa.

Specific Activity (Parent lot# 1606088): 246U/mg, where one unit of MRCK α , active 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.

Formulation: 1.188mg/ml of enzyme in of 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.

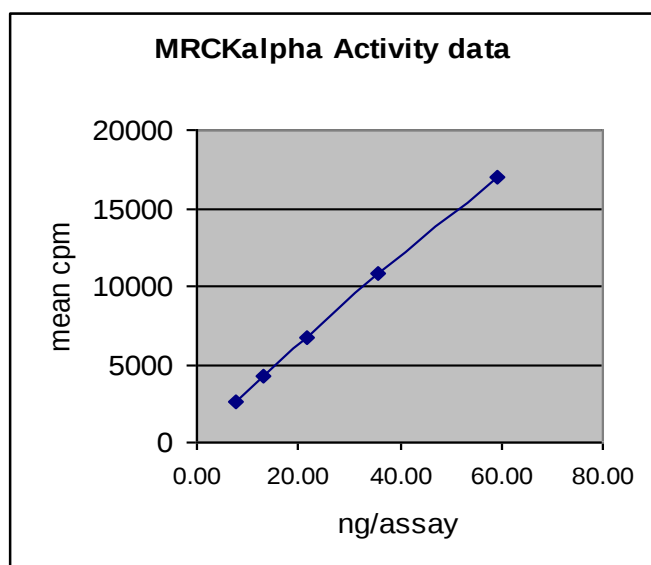
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

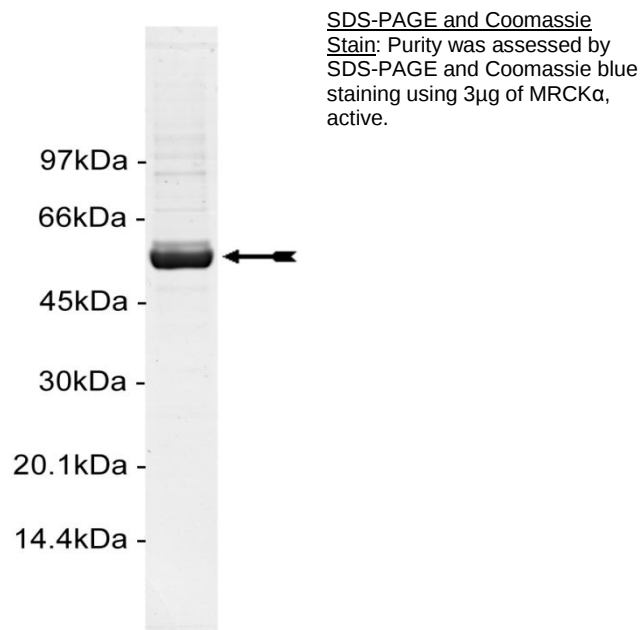
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 7.8–59.4ng 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 product identity as MRCK α 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. **Substrate peptide (KKRNRTLTV):** Use at a final assay concentration of 100 μ M. Make up a 1mM stock. Add 2.5 μ l of stock per assay point.
3. **MRCK α , active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 7.8–59.4ng 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:

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of substrate peptide (**KKRNRTLTV**).
3. Add **2.5 μ l (7.8–59.4ng) MRCK α , active.**
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]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.

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MRCK α Sequence Information

Protein	Human MRCK α
Tags	C-terminal 6His
Native sequence	M1 of recombinant protein is equivalent to M1 of human MRCK α
Accession number	GenBank NM_003607

Recombinant MRCK α amino acid sequence:

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1 MSGEVRLRQL EQFILDGPAQ TNGQCFSVET LLDILICLYD ECNNSPLRRE KNILEYLEWA
61 KPFTSKVKQM RLHREDFEIL KVIGRGAFGE VAVVKLKNAD KVFAMKILNK WEMLKRAETA
121 CFREERDVLV NGDNKWITTL HYAFQDDNNL YLVMDYYVGG DLLTLLSKFE DRLPEDMARF
181 YLAEMVIAID SVHQLHYVHR DIKPDNILMD MNGHIRLADF GSCLKLMEDG TVQSSVAVGT
241 PDYISPEILQ AMEDGKGRYG PECDWWSLGV CMYEMLYGET PFYAESLVET YGKIMNHKER
301 FQFPAQVTDV SENAKDLIRR LICSRHRLG QNGIEDFKKH PFFSGIDWDN IRNCEAPYIP
361 EVSSPTDTSN FDVDDCLKN SETMPPPTH AFSGHLPFV GFTYTSSCVL SDRSCLRVTA
421 GPTSLDLVDN VQRTLDNNLA TEAYERRIKR LEQEKLELSR KLQESTQTVQ ALQHHHHHH

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Recombinant MRCK α nucleotide sequence:

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1 atgtctggag aagtgcgttt gaggcagttg gagcagttta ttttggacgg gcccgcctcag
61 accaatgggc agtgcttcag tgtggagaca ttactggata tactcatctg cctttatgat
121 gaatgcaata attctccatt gagaagagag aagaacattc tcgaatacct agaatgggct
181 aaaccattta cttctaaagt gaaacaaatg cgattacata gagaagactt tgaaatatta
241 aaggtgattg gtcgaggagc ttttggggag gttgctgtag taaaactaaa aaatgcagat
301 aaagtgtttg ccatgaaaat attgaataaa tgggaaatgc tgaaaagagc tgagacagca
361 tgttttcgtg aagaaaggga tgtattagtg aatggagaca ataaatggat tacaaccttg
421 cactatgctt tccaggatga caataactta tacctgggta tggattatta tgttggtggg
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1381 aaacttcaag agtcaacaca gactgtccaa gctctgcagc atcaccatca ccatcactaa

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