

Certificate of Analysis

MRCK γ , active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 16-046, 16-046-K, 16-046M

Parent Lot # D17NP005N

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 FLAG-tagged and N-terminal 6His-tagged, recombinant, human MRCK γ amino acids 1-473 expressed by baculovirus in Sf21 insect cells. Purified using immunoaffinity chromatography.

Purity 99% by SDS-PAGE and Coomassie blue staining. MW = 56kDa.

Specific Activity (Parent lot# D17NP005N): 193U/mg, where one unit of MRCK γ activity is defined as 1nmol phosphate incorporated into 50 μ M KKRNRTLTV per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.58mg/ml of enzyme in 43mM Tris/HCl pH7.5, 129mM NaCl, 270mM sucrose, 129 μ g/ml FLAG peptide, 0.9mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

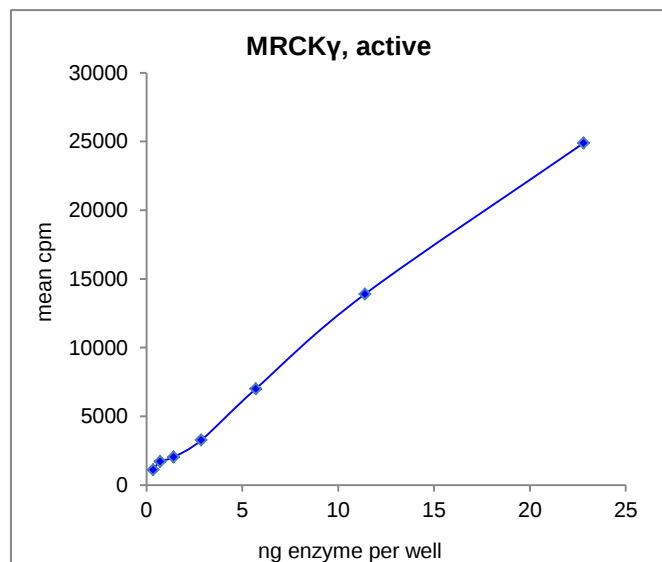
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

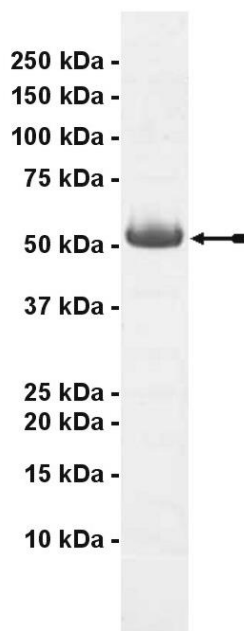
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1–23ng of this lot of enzyme phosphorylated 50 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 γ with the translated 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 γ , active.

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. KKRNRTLTV:** Use at a final assay concentration of 50 μ M. Prepare a 1mM stock and add 1.25 μ l of stock per assay point.
- 3. MRCK γ , active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1–23ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 1.25 μ l of KKRNRTLTV.
3. Add **2.5 μ l (1–23ng) MRCK γ , active.**
4. Add 6.25 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 30 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 dried 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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MRCKy, active Sequence Information

Protein	Human MRCKy
Tags	N-terminal FLAG and N-terminal 6His
Native sequence	M20 of the recombinant protein is equivalent to M1 of human MRCKy.
Accession number	GenPept NP_059995.1 (synthetic coding sequence)

Recombinant MRCKy amino acid sequence:

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1  MGGDYKDDDD KDPHHHHHMM ERRRLALEQL ARGEAGGCPG LDGLLDLLLA LHHELSSGPL
61  RRERSVAQFL SWASPFVSKV KELRLQRDDF EILKVIKRG FGEVTVVRQR DTGQIFAMKM
121 LHKWEMLKRA ETACFREERD VLVKGDNRWV TTLHYAFQDE EYLYLVMDYY AGDLLTLLS
181 RFEDRLPPEL AQFYLAEMVL AIHSLHQLGY VHRDVKPDNV LLDVNGHIRL ADFGSLRLN
241 TNGMVDSSVA VGTPDYISPE ILQAMEEGKG HYGPQCDWWS LGVCAYELLF GETPFYAESL
301 VETYGKIMNH EDHLQFPDPV PDVPASAQDL IRQLLCRQEE RLGRGGLDDF RNHPFFEGVD
361 WERLASSTAP YIPELRGPM DTSNFDVDDDT LNHPGTLPPP SHGAFSGHHL PFVGFYTYTSG
421 SHSPESSEA WAALERKLQC LEQEKVELSR KHQEALHAPT DHRELEQLRK EVQTLRDLRP
481 EMLRDKASLS QT

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Recombinant MRCKy nucleotide sequence:

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1  atgggaggtg actacaaaga cgatgacgac aaggatccac atcaccatca ccatcacatg
61  gaaagaaggt tgagggctct ggaacaattg gcaaggggag aagccggtgg ctgccagggc
121 ctggatggac tcctggacct gctgctggca ctgcaccacg aactgtcctc tggacccttg
181 aggagggaga ggtccgttgc tcagttcctg tcctgggcat caccttttgt ctctaagggt
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1441 gagatgttga gggataaagc ctcattgagc caaacctaa

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