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Certificate of Analysis

MRCKγ, 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 MRCKγ 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 MRCKγ activity is defined as 1nmol phosphate incorporated into 50µM KKRNRTLTV per minute at 30°C with a final ATP concentration of 100µM.

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

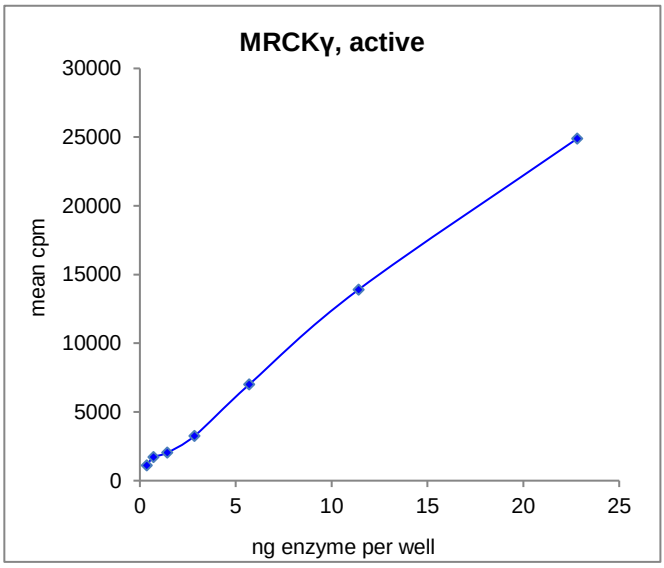
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

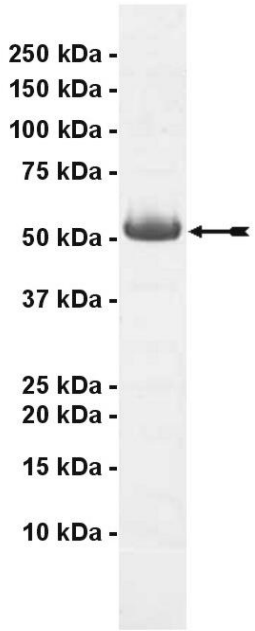
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.4–22.8ng of this lot of enzyme phosphorylated 50µM 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 MRCKγ with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of MRCKγ, 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 50µM. Prepare a 1mM stock and add 1.25µl of stock per assay point.
3. **MRCKγ, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.4–22.8ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x MgAc/[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 1.25µl of KKRNRTLTV.
3. Add **2.5µl (0.4-22.8ng) MRCKγ, active.**
4. Add 6.25µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 30 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 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 1µl of 30% phosphoric acid.

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MRCKγ, active Sequence Information

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

Recombinant MRCKγ amino acid sequence:

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   1 MGGDYKDDDD KDPHHHHHHM ERRLRALEQL ARGEAGGCPG LDGLLDLLLA LHHELSSGPL
  61 RRERSVAQFL SWASPFVSKV KELRLQRDDF EILKVIGRGA FGEVTVVRQR DTGQIFAMKM
 121 LHKWEMLKRA ETACFREERD VLVKGDSRWV TTLHYAFQDE EYLYLVMDYY AGGDLLTLLS
 181 RFEDRLPPEL AQFYLAEMVL AIHSLHQLGY VHRDVKPDNV LLDVNGHIRL ADFGSCLRLN
 241 TNGMVDSSVA VGTPDYISPE ILQAMEEGKG HYGPQCDWWS LGVCAYELLF GETPFYAESL
 301 VETYGKIMNH EDHLQFPPDV PDVPASAQDL IRQLLCRQEE RLGRGGLDDF RNHPFFEGVD
 361 WERLASSTAP YIPELRGPMD TSNFDVDDDT LNHPGTLPPP SHGAFSGHHL PFVGFTYTSG
 421 SHSPESSSEA WAALERKLQC LEQEKVELSR KHQEALHAPT DHRELEQLRK EVQTLRDRLP
 481 EMLRDKASLS QT
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Recombinant MRCKγ nucleotide sequence:

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   1 atgggaggtg actacaaaga cgatgacgac aaggatccac atcaccatca ccatcacatg
  61 gaaagaaggt tgagggctct ggaacaattg gcaaggggag aagccggtgg ctgcccaggc
 121 ctggatggac tcctggacct gctgctggca ctgcaccacg aactgtcctc tggacccttg
 181 aggagggaga ggtccgttgc tcagttcctg tcctgggcat cacctttttgt ctctaaggtt
 241 aaggaactgc gcctccagcg cgatgatttc gagatcctga aagtgattgg taggggtgct
 301 tttggtgaag ttactgtcgt gcgccagcgc gataccggcc aaattttcgc catgaagatg
 361 ctccacaaat gggaaatgct caagagggcc gaaaccgcct gcttccgcga agagcgcgat
 421 gttctcgtta aaggcgattc aaggtgggtt acaaccttgc attacgcctt tcaggacgag
 481 gagtacctgt atctggtcat ggactattac gccggaggtg acctcctgac tttgctcagc
 541 cgcttcgaag acaggctgcc tcccgagctc gctcaatttt acctggctga aatggttctg
 601 gccattcata gcctccacca gctgggatat gtccatcgcg atgtgaaacc agacaatgtc
 661 ctgttggacg tgaacggcca cattagactc gccgatttcg gtagctgcct gcgcttgaat
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 781 attctgcaag caatggagga aggaaaagga cactacggcc cccaatgcga ctggtggtct
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1021 cgcttgggcc gcggcggcct ggacgatttc aggaatcacc ccttctttga gggagtcgat
1081 tgggaaaggc tcgcttcaag caccgcccca tacatcccag agctgagggg acccatggac
1141 acctccaatt tcgatgtgga tgacgacact ctgaaccacc caggcacctt gccacccccc
1201 agccacggcg ccttctccgg ccaccatctg ccattcgttg gctttactta cacctccgga
1261 agccacagcc cagaatcttc ctctgaggct tgggctgcct tggagaggaa attgcagtgc
1321 ctggaacaag agaaggtcga actgagccgc aagcatcagg aagctttgca cgctcccact
1381 gaccataggg aactggaaca actgaggaag gaagtgcaaa ctttgcgcga tagactgcct
1441 gagatgttga gggataaagc ctcattgagc caaacctaa
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Reviewed and approved by site quality representative.