

Catalogue #	Aliquot Size
C27-11G-05	5 µg
C27-11G-10	10 µg
C27-11G-20	20 µg

MRCK α , Active

Recombinant human protein expressed in Sf9 cells

Catalog # C27-11G

Lot # N331-2

Product Description

Recombinant human MRCK α (1-473) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_003607](#).

Gene Aliases

CDC42BPA, MRCK, PK428, FLJ23347, KIAA0451, DKFZp686L1738, DKFZp686P1738

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

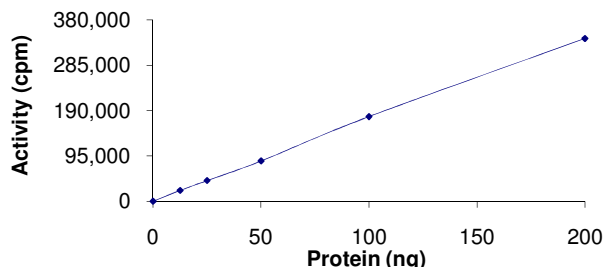
Scientific Background

Myotonic Dystrophy Kinase-Related Cdc42-Binding Kinase α (MRCK α) is a Cdc42/Rac/Rho interactive/binding serine/threonine kinase with multiple functional domains (1). MRCK are effectors of RhoA and Cdc42, respectively, for actin reorganization. MRCK α is a critical regulator of signal transduction pathways in eukaryotic cells that are known principally for their role in regulating the cytoskeleton, and they do so by recruiting a variety of downstream effector proteins (2).

References

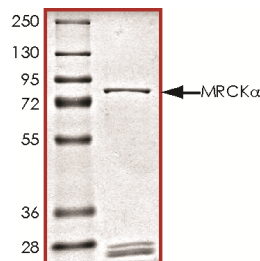
- Ivan, T. et al: Genomic organization of human myotonic dystrophy kinase-related Cdc42-binding kinase α reveals multiple alternative splicing and functional diversity. *Gene*; 2003; 304:107-115.
- Ivan, T. et al: Phosphorylation of a Novel Myosin Binding Subunit of Protein Phosphatase 1 Reveals a Conserved Mechanism in the Regulation of Actin Cytoskeleton. *J. Biol. Chem.*, 2001; 276 (24): 21209-21216.

Specific Activity



The specific activity of MRCK α was determined to be **86 nmol /min/mg** as per activity assay protocol.

Purity



The purity of MRCK α was determined to be **>70%** by densitometry, approx. MW **73kDa**.

MRCK α , Active

Recombinant human protein expressed in Sf9 cells

Catalog Number	C27-11G
Specific Activity	86 nmol/min/mg
Lot Specific Number	N331-2
Purity	>70%
Concentration	0.1 µg/µl
Stability	1yr At -70°C from date of shipment
Storage & Shipping	Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: C27-11G)

Active MRCK α (0.1 μ g/ μ l) diluted with Kinase Dilution Buffer (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active MRCK α for optimal results).

Kinase Dilution Buffer (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/ μ l BSA solution.

Kinase Assay Buffer (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β -glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250 μ M [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 μ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 μ l [³³P]-ATP (1mCi/100 μ l), 5.75ml of Kinase Assay Buffer (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer (Catalog #: K01-09). Store 200 μ l aliquots at -20°C.

Substrate (Catalog #: S05-58)

S6K synthetic peptide substrate (KRRRLASLR) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active MRCK α , Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 μ l:
 - Component 1.** 10 μ l of diluted Active MRCK α (Catalog #C27-11G)
 - Component 2.** 5 μ l of 1mg/ml stock solution of substrate (Catalog #S05-58)
 - Component 3.** 5 μ l distilled H₂O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5.** Initiate the reaction by the addition of 5 μ l [³³P]-ATP Assay Cocktail bringing the final volume up to 25 μ l and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 μ l of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μ l [³³P]-ATP / pmoles of ATP (in 5 μ l of a 250 μ M ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/ μ g or nmol/min/mg)

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μ g or mg)]*[(Reaction Volume) / (Spot Volume)]

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