BioVision
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Active MRCKβ

Recombinant protein expressed in Sf9 cells

Catalog # 7772-5 ***Lot #*** _____

Product Description

Recombinant human MRCKβ (1-473) was expressed by baculovirus in Sf9 insect cells using an N-terminal tag. The gene accession number is NM_006035.

Gene Aliases

CDC42BPB, KIAA1124

Formulation

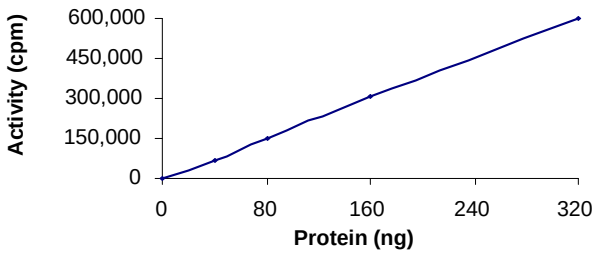
Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

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.

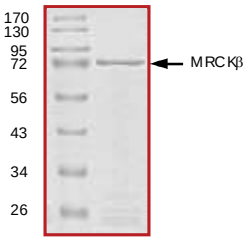
Scientific Background

Myotonic Dystrophy Kinase-Related cdc42-binding kinase beta (MRCKβ) belongs to the DMPK subfamily (1). The myotonic dystrophy kinase-related kinases and myotonic dystrophy kinase-related Cdc42 binding kinase (MRCK) are effectors of RhoA and Cdc42, respectively, where they are involved in actin cytoskeletal reorganization and neurite outgrowth (2). Effects of the repeat expansion on the DMPK gene may be responsible for muscle and heart features of Myotonic Dystrophy.



The specific activity of MRCKB was determined to be **120 nmol / min/ mg** as per activity assay protocol.

Purity



The purity of MRCKβ was determined to be **>90%** by densitometry, approx. MW **73kDa**.

Active MRCKβ

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	7772-5
Quantity	5µg
Specific Activity	120 nmol
Lot Specific Number	_____
Purity	>90%
Format	5µg in 50µl

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DMPK, is expressed almost exclusively in muscle and heart. Hum. Mol. Genet. 2000; 9(14): 2167-73.

2. Tan, I. 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):21209-16.

Specific Activity

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.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: 7758-5)

Active MRCKβ (0.1μg/μl) diluted with Kinase Dilution Buffer 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 MRCKβ for optimal results).

Kinase Dilution Buffer

Kinase Assay Buffer I diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA solution.

[^{32}P]-ATP Assay Cocktail

Prepare 250μM [^{32}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution, 100μl [^{32P}]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer. Store 1ml aliquots at –20°C.

10mM ATP Stock Solution

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer. Store 200μl aliquots at –20°C.

Kinase Assay Buffer

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

Substrate

S6K synthetic peptide substrate (KRRRLASLR) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{32}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active MRCKβ, 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 20μl:

- **Component 1.** 10μl of diluted Active MRCKβ
- **Component 2.** 5μl of 1mg/ml stock solution of substrate
- **Component 3.** 5μl distilled H_2O (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 H_2O.

Step 5. Initiate the reaction by the addition of 5μl [^{32}P]-ATP Assay Cocktail bringing the final volume up to 25μl 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 20μl 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 H_2O) 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.

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

Corrected cpm from reaction / [(SA of ^{32}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]