Entry Clone Source: MGC

Entry Clone Accession: IMAGE:4139392

SGC Construct ID: DYRK2A-c022

GenBank GI number: gi|4503427

Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]

Coding DNA sequence:

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CATATGCACCATCATCATCATCATTCTTC
TGGTGTAGATCTGGGTACCGAGAACCTGT
ACTTCCAATCCATGGGGAAGGTGAAAGCC
ACCCCCATGACACCTGAACAAGCAATGAA
GCAATACATGCAAAAACTCACAGCCTTCG
AACACCATGAGATTTTCAGCTACCCTGAA
ATATATTTCTTGGGTCTAAATGCTAAGAA
GCGCCAGGGCATGACAGGTGGGCCCAACA
ATGGTGGCTATGATGATGACCAGGGATCA
TATGTGCAGGTGCCCCACGATCACGTGGC
TTACAGGTATGAGGTCCTCAAGGTCATTG
GGAAGGGGAGCTTTGGGCAGGTGGTCAAG
GCCTACGATCACAAAGTCCACCAGCACGT
GGCCCTAAAGATGGTGCGGAATGAGAAGC
GCTTCCACCGGCAAGCAGCGGAGGAGATC
CGAATCCTGGAACACCTGCGGAAGCAGGA
CAAGGATAACACAATGAATGTCATCCATA
TGCTGGAGAATTTCACCTTCCGCAACCAC
ATCTGCATGACGTTTGAGCTGCTGAGCAT
GAACCTCTATGAGCTCATCAAGAAGAATA
AATTCCAGGGCTTCAGTCTGCCTTTGGTT
CGCAAGTTTGCCCACTCGATTCTGCAGTG
CTTGGATGCTTTGCACAAAAACAGAATAA
TTCACTGTGACCTTAAGCCCGAGAACATT
TTGTTAAAGCAGCAGGGTAGAAGCGGTAT
TAAAGTAATTGATTTTGGCTCCAGTTGTT
ACGAGCATCAGCGTGTCTACACGTACATC
CAGTCGCGTTTTTACCGGGCTCCAGAAGT
GATCCTTGGGGCCAGGTATGGCATGCCCA
TTGATATGTGGAGCCTGGGCTGCATTTTA
GCAGAGCTCCTGACGGGTTACCCCCTCTT
GCCTGGGGAAGATGAAGGGGACCAGCTGG
CCTGTATGATTGAACTGTTGGGCATGCCC
TCACAGAAACTGCTGGATGCATCCAAACG
AGCCAAAAATTTTGTGAGCTCCAAGGGTT
ATCCCCGTTACTGCACTGTCACGACTCTC
TCAGATGGCTCTGTGGTCCTAAACGGAGG
CCGTTCCCGGAGGGGGAAACTGAGGGGCC
CACCGGAGAGCAGAGAGTGGGGGAACGCG
CTGAAGGGGTGTGATGATCCCCTTTTCCT
TGACTTCTTAAAACAGTGTTTAGAGTGGG
ATCCTGCAGTGCGCATGACCCCAGGCCAG
GCTTTGCGGCACCCCTGGCTGAGGAGGCG
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GTTGCCAAAGCCTCCCACCGGGGAGAAAA
CGTCAGTGAAAAGGTGACAGTAAAGGTGG
ATACGGATCCGAA

Tags and additions: Cleavable N-terminal His6 tag.

Host: BL21 (DE3)R3-pRARE2 (Phage resistant strain)

Expressed protein sequence (tag sequence in lowercase):
mhhhhhhssgvdlgtenlyfq^sMGKVKA
TPMTPEQAMKQYMQKLTAFEHHEIFSYPE
IYFLGLNAKKRQGMTGGPNNGGYDDDQGS
YVQVPHDHVAYRYEVLKVIGKGSFGQVVK
AYDHKVHQHVALKMVRNEKRFHRQAAEEI
RILEHLRKQDKDNTMNVIHMLENFTFRNH
ICMTFELLSMNLYELIKKNKFQGFSLPLV
RKFAHSILQCLDALHKNRIIHCDLKPENI
LLKQQGRSGIKVIDFGSSCYEHQRVYTYI
QSRFYRAPEVILGARYGMPIDMWSLGCIL
AELLTGYPLLPGEDEGDQLACMIELLGMP
SQKLLDASKRAKNFVSSKGYPRYCTVTTL
SDGSVVLNGGRSRRGKLRGPPESREWGNA
LKGCDDPLFLDFLKQCLEWDPAVRMTPGQ
ALRHPWLRRRLPKPPTGEKTSVKR

^ TEV cleave site

Growth medium, induction protocol: 5 ml from a 50 ml overnight culture containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol were used to inoculate each of two 1 litre cultures of LB containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol. Cultures were grown at 37°C until the OD_{600} reached ~0.5 then the temperature was adjusted to 18°C. Expression was induced overnight using 0.5 mM IPTG at an OD_{600} of 0.9. The cells were collected by centrifugation and the pellet re-suspended in binding buffer and frozen. **Binding buffer:** 50 mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5 % glycerol.

Extraction buffer, extraction method: Frozen pellets were thawed and fresh 0.5 mM TCEP added to the lysate. Cells were lysed using sonication. The lysate was centrifuged at 16,500 rpm for 60 minutes and the supernatant collected for purification.

Column 1: Ni-affinity. Ni-sepharose (Amersham), 5 ml of 50 % slurry in 1.5 x 10 cm column, washed with binding buffer.

Buffers:
Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, 5% Glycerol
Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 20 mM Imidazole, 5% glycerol
Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 50 to 250 mM Imidazole , 5% Glycerol (step elution).

Procedure: The lysate supernatant was loaded by gravity flow on the Ni-sepharose column. The column was then washed with 30 ml wash buffer at gravity flow. The protein was eluted by gravity flow by applying 5-ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150 and 250 mM); fractions were collected until essentially all protein was eluted.

Column 2: Size Exclusion Chromatography. Superdex S200 16/60 HiLoad

Buffers: 25 mM HEPES, pH 7.5; 500 mM NaCl, 0.5 mM TCEP
Procedure: The protein was concentrated and applied to an S200 16/60 HiLoad gel filtration column equilibrated in 25 mM HEPES, pH 7.5; 500 mM NaCl, 0.5 mM TCEP using an ÄKTAexpress system.
Mass spectrometry characterization: LC- ESI -MS TOF showed that the protein was herterogeneously phosphorylated at up to 4 sites in accordance with a mass of 49279 for this construct as predicted from the sequence of this protein.
Protein concentration: Protein was concentrated to 7.3 mg/ml using an Amicon 10 kDa cut-off concentrator.
Crystallization: Crystals were grown at 4°C in 300 nl sitting drops from a 2:1 ratio of protein to reservoir solution containing 0.2M Na(ac); 0.1 M cacodylate pH 6.5; 30% PEG 8K.
Data Collection: Crystals were cryo-protected using the well solution supplemented with 2M Li_2SO_4 and flash frozen in liquid nitrogen. **X-ray source:** Diffraction data were collected from a single crystal on Diamond beamline IO3 at a single wavelength of 0.9763 Å and the structure was refined to 2.2 Å. **Phasing:** The structure was solved by molecular replacement using the structure of human DYRK2 (PDB ID 3K2L) as a starting model.