DYRK2A (4AZF) Materials & Methods

Entry Clone Source: Mammalian Gene Collection (IMAGE Consortium Clone ID 4139392)

SGC Construct ID: DYRK2A-c023

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

DNA sequence:

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ATGCACCATCATCATCATCATTCTTC
TGGTGTAGATCTGGGTACCGAGAACC
TGTACTTCCAATCCATGGGGAAGGTG
AAAGCCACCCCCATGACACCTGAACA
AGCAATGAAGCAATACATGCAAAAAC
TCACAGCCTTCGAACACCATGAGATT
TTCAGCTACCCTGAAATATATTTCTT
GGGTCTAAATGCTAAGAAGCGCCAGG
GCATGACAGGTGGGCCCAACAATGGT
GGCTATGATGATGACCAGGGATCATA
TGTGCAGGTGCCCCACGATCACGTGG
CTTACAGGTATGAGGTCCTCAAGGTC
ATTGGGAAGGGGAGCTTTGGGCAGGT
GGTCAAGGCCTACGATCACAAAGTCC
ACCAGCACGTGGCCCTAAAGATGGTG
CGGAATGAGAAGCGCTTCCACCGGCA
AGCAGCGGAGGAGATCCGAATCCTGG
AACACCTGCGGAAGCAGGACAAGGAT
AACACAATGAATGTCATCCATATGCT
GGAGAATTTCACCTTCCGCAACCACA
TCTGCATGACGTTTGAGCTGCTGAGC
ATGAACCTCTATGAGCTCATCAAGAA
GAATAAATTCCAGGGCTTCAGTCTGC
CTTTGGTTCGCAAGTTTGCCCACTCG
ATTCTGCAGTGCTTGGATGCTTTGCA
CAAAAACAGAATAATTCACTGTGACC
TTAAGCCCGAGAACATTTTGTTAAAG
CAGCAGGGTAGAAGCGGTATTAAAGT
AATTGATTTTGGCTCCAGTTGTTACG
AGCATCAGCGTGTCTACACGTACATC
CAGTCGCGTTTTTACCGGGCTCCAGA
AGTGATCCTTGGGGCCAGGTATGGCA
TGCCCATTGATATGTGGAGCCTGGGC
TGCATTTTAGCAGAGCTCCTGACGGG
TTACCCCCTCTTGCCTGGGGAAGATG
AAGGGGACCAGCTGGCCTGTATGATT
GAACTGTTGGGCATGCCCTCACAGAA
ACTGCTGGATGCATCCAAACGAGCCA
AAAATTTTGTGAGCTCCAAGGGTTAT
CCCCGTTACTGCACTGTCACGACTCT
CTCAGATGGCTCTGTGGTCCTAAACG
GAGGCCGTTCCCGGAGGGGGAAACTG
AGGGGCCCACCGGAGAGCAGAGAGTG
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GGGGAACGCGCTGAAGGGGTGTGATG
ATCCCCTTTTCCTTGACTTCTTAAAA
CAGTGTTTAGAGTGGGATCCTGCAGT
GCGCATGACCCCAGGCCAGGCTTTGC
GGCACCCCTGGCTGAGGAGGCGGTTG
CCATGA

Final protein sequence (Tag sequence in lowercase):
mhhhhhhssgvdlgtenlyfq^smGK
VKATPMTPEQAMKQYMQKLTAFEHHE
IFSYPEIYFLGLNAKKRQGMTGGPNN
GGYDDDQGSYVQVPHDHVAYRYEVLK
VIGKGSFGQVVKAYDHKVHQHVALKM
VRNEKRFHRQAAEEIRILEHLRKQDK
DNTMNVIHMLENFTFRNHICMTFELL
SMNLYELIKKNKFQGFSLPLVRKFAH
SILQCLDALHKNRIIHCDLKPENILL
KQQGRSGIKVIDFGSSCYEHQRVYTY
IQSRFYRAPEVILGARYGMPIDMWSL
GCILAELLTGYPLLPGEDEGDQLACM
IELLGMPSQKLLDASKRAKNFVSSKG
YPRYCTVTTLSDGSVVLNGGRSRRGK
LRGPPESREWGNALKGCDDPLFLDFL
KQCLEWDPAVRMTPGQALRHPWLRRR
LP

(Met73 to Pro467)

^ TEV protease recognition site

Tags and additions: Cleavable N-terminal His6 tag

Expression: The DYRK2 construct was transformed into BL21(DE3)-R3-pRARE2 cells and expressed in LB media containing 50 µg/ml kanamycin.

Cell Harvest: Cells were spun at 5000x g for 20 mins and the pellets resuspended in Lysis Buffer and then frozen at -20°C.

Cell lysis: The resuspended cell pellet was thawed and lysed by high pressure homogenisation. The cell debris and precipitated DNA were spun down.
Lysis buffer: 50 mM Hepes pH 7.5, 500 mM NaCl, 5 mM Imidazole, 5% glycerol, 0.5 mM TCEP

Column 1: 5 ml of Ni-Sepharose in a gravity flow column.

Column 1 Buffers:
Binding buffer: 50 mM Hepes pH 7.5, 500 mM NaCl, 5 mM Imidazole, 5% glycerol, 0.5 mM TCEP
Wash buffer: As Binding Buffer except 25 mM imidazole.
Elution buffer: As Binding Buffer except 50, 100, 150, 200, 250 mM imidazole.

Column 1 Procedure: The clarified supernatant was passed through the column. The column was washed with 100 ml of Binding Buffer and 100 ml of Wash Buffer. 5 ml fractions of each step of Elute Buffer were passed through to elute the protein.

Column 2: S200 16/60 Gel Filtration (GE Healthcare)
Column 2 Buffers: **Gel Filtration buffer:** 50 mM Hepes pH 7.5, 500 mM NaCl, 0.5 mM TCEP
Column 2 Procedure: The eluted protein was concentrated to 5 ml volume and injected onto the column.
Concentration: Crystals grew from a 2:1 ratio of protein and precipitant solution (0.2M Na/KPO_4, 20% PEG 3350, 10% Ethylene Glycol), using the vapour diffusion method.
Data Collection: Crystals were cryo-protected by equilibration into precipitant solution containing 25% ethylene glycol, and then flash frozen in liquid nitrogen. Data was collected at Diamond, beamline IO2.