MAPK7A (4B99) Materials & Methods

Entry Clone Source: Mammalian Gene Collection (IMAGE Consortium Clone ID 4111084).

SGC Construct ID: MAPK7A-c029

Vector: pFB-LIC-Bse. Details [PDF]; Sequence [FASTA] or [GenBank]

DNA sequence:

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ATGGGCCACCATCATCATCATCATTCTTC
TGGTGTAGATCTGGGTACCGAGAACCTGT
ACTTCCAATCCATGGCCGAGCCTCTGAAG
GAGGAAGACGGCGAGGACGGCTCTGCGGA
GCCCCCCGGGCCCGTGAAGGCCGAACCCG
CCCACACCGCTGCCTCTGTAGCGGCCAAG
AACCTGGCCCTGCTTAAAGCCCGCTCCTT
CGATGTGACCTTTGACGTGGGCGACGAGT
ACGAGATCATCGAGACCATAGGCAACGGG
GCCTATGGAGTGGTGTCCTCCGCCCGCCG
CCGCCTCACCGGCCAGCAGGTGGCCATCA
AGAAGATCCCTAATGCTTTCGATGTGGTG
ACCAATGCCAAGCGGACCCTCAGGGAGCT
GAAGATCCTCAAGCACTTTAAACACGACA
ACATCATCGCCATCAAGGACATCCTGAGG
CCCACCGTGCCCTATGGCGAATTCAAATC
TGTCTACGTGGTCCTGGACCTGATGGAAA
GCGACCTGCACCAGATCATCCACTCCTCA
CAGCCCCTCACACTGGAACACGTGCGCTA
CTTCCTGTACCAACTGCTGCGGGGCCTGA
AGTACATGCACTCGGCTCAGGTCATCCAC
CGTGACCTGAAGCCCTCCAACCTATTGGT
GAATGAGAACTGTGAGCTCAAGATTGGTG
ACTTTGGTATGGCTCGTGGCCTGTGCACC
TCGCCCGCTGAACATCAGTACTTCATGAC
TGAGTATGTGGCCACGCGCTGGTACCGTG
CGCCCGAGCTCATGCTCTCTTTGCATGAG
TATACACAGGCTATTGACCTCTGGTCTGT
GGGCTGCATCTTTGGTGAGATGCTGGCCC
GGCGCCAGCTCTTCCCAGGCAAAAACTAT
GTACACCAGCTACAGCTCATCATGATGGT
GCTGGGTACCCCATCACCAGCCGTGATTC
AGGCTGTGGGGGCTGAGAGGGTGCGGGCC
TATATCCAGAGCTTGCCACCACGCCAGCC
TGTGCCCTGGGAGACAGTGTACCCAGGTG
CCGACCGCCAGGCCCTATCACTGCTGGGT
CGCATGCTGCGTTTTGAGCCCAGCGCTCG
CATCTCAGCAGCTGCTGCCCTTCGCCACC
CTTTCCTGGCCAAGTACCATGATCCTGAT
GATGAGCCTGACTGTGCCCCGCCCTTTGA
CTTTGCCTTTGACCGCGAAGCCCTCACTC
GGGAGCGCATTAAGGAGGCCATTGTGGCT
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GAAATTGAGGACTTCCATGCAAGGCGTGA
GGGCATCCGCCAATGA

Final protein sequence (Tag sequence in lowercase):
mghhhhhhssgvdlgtenlyfq^smA
EPLKEEDGEDGSAEPPGPVKAEPAHT
AASVAAKNLALLKARSFDVTFDVGDE
YEIIETIGNGAYGVVSSARRRLTGQQ
VAIKKIPNAFDVVTNAKRTLRELKIL
KHFKHDNIIAIKDILRPTVPYGEFKS
VYVVLDLMESDLHQIIHSSQPLTLEH
VRYFLYQLLRGLKYMHSAQVIHRDLK
PSNLLVNENCELKIGDFGMARGLCTS
PAEHQYFMTEYVATRWYRAPELMLSL
HEYTQAIDLWSVGCIFGEMLARRQLF
PGKNYVHQLQLIMMVLGTPSPAVIQA
VGAERVRAYIQSLPPRQPVPWETVYP
GADRQALSLLGRMLRFEPSARISAAA
ALRHPFLAKYHDPDDEPDCAPPFDFA
FDREALTRERIKEAIVAEIEDFHARR
EGIRQ

^ TEV protease recognition site (Met1 to Gln397)

Tags and additions: Cleavable N-terminal His6 tag

Expression: The MAPK7 protein was expressed using baculovirus infected Sf9 cells. Cells were infected at a density of 2,000,000 cells/ml for 48h.

Cell harvest: Cells were spun at 1000x g for 20 mins and the pellets resuspended in Lysis Buffer and then frozen at -20°C.

Purification

Cell Lysis: The resuspended cell pellet was thawed and lysed by sonication. PEI (polyethyleneimine) was added to a final concentration of 0.15 %. The cell debris and precipitated DNA were spun down.
Lysis buffer: 50 mM Tris pH 7.8, 200 mM NaCl, 20 mM Imidazole, 0.5 mM TCEP, 1:2000 dilution of Sigma protease inhibitor cocktail.

Column 1: 6 ml of Ni-Sepharose in a 2 cm diameter gravity flow column

Column 1 Buffers:
Binding buffer: 50 mM Tris pH 7.8, 200 mM NaCl, 20 mM Imidazole, 0.5 mM TCEP.
Wash buffer 1: As Binding Buffer except 1 M NaCl and 40 mM imidazole.
Wash buffer 2: As Binding Buffer except 60 mM imidazole.
Elution buffer: As Binding Buffer except 250 mM imidazole

Column 1 Procedure: The clarified supernatant was passed through the column. The column was washed with 50 ml of Binding Buffer (Wash 1) and 50 ml of Wash Buffer 1 (Wash 2) and 40 ml of Wash Buffer 2 (Wash 3). 36 ml of Elute Buffer was passed through to elute the protein.
The Wash 3 and Elute fractions were combined and TEV protease was added, The sample was left at 4°C overnight.

Column 2: S200 16/60 Gel Filtration (GE Healthcare)
Column 2 Buffers: **Gel filtration buffer:** 20 mM Tris pH 7.8, 200 mM NaCl, 0.5 mM TCEP
Column 2 Procedure: The eluted protein was concentrated to 5 ml volume and injected onto the column
Column 3: 1 ml of Ni-Sepharose in a 0.5 cm diameter gravity flow column
Column 3 Procedure: The pooled fractions from gel filtration were passed through the column (pre-equilibrated in GF Buffer) followed by 5 ml of addition GF Buffer. The resin was eluted with 5 ml of GF Buffer containing 10 mM, 20 mM, 30 mM and finally 40 mM imidazole.
Column 4: S200 16/60 Gel Filtration (GE Healthcare)
Column 4 Buffers: **Gel filtration buffer:** 20 mM Tris pH 7.8, 200 mM NaCl, 0.5 mM TCEP
Column 4 Procedure: The flow-through, and 10 mM and 20 mM elutions from column 3 were combined, concentrated, and injected onto the column.
Concentration: The fractions containing MAPK7 were combined. Compound K03750 (XMD17-26) was added and the sample concentrated to 12.6 mg/ml (measured by 280 nm absorbance).
Mass spec characterization: Expected: 44942.7 Observed: 44943.7
Crystallization: Crystals grew in 150 nL drops from a 1:1 ratio of protein and precipitant solution (0.05M CaCl2, 0.1M MES pH 6.0, 20% PEG 6000, 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 I24.