

Molecular Biology

Entry Clone Accession:

Entry Clone Source: Tony Pawson

SGC Construct ID: WNK3B-c005

Protein Region: E132-E414

Vector: pFB-LIC-Bse

Tag: N-6HIS;N-TEV

Host: DH10Bac

Sequence (with tag(s)):

MGHHHHHHSSGVDLGTENLYFQSMEAEMKAVATSPSGRFLKFDIELGRGAFKTVYKGLD
TETWVEVAWCELQDRKLTAEQQRKFKEEAEMLKGLQHPNIVRFYDSWESILKGKKCIVL
VTELMTSGTLKTYLKRFKVMKPKVLRSWCRQILKGLQFLHTRTPPIIHRDLKCDNIFITGP
TGSVKIGDLGLATLMRTSFAKSVIGTPEFMAPEMYEEHYDESVDVYAFGMCMLEMATSE
YPYSECQNAAQIYRKVTSGIKPASFNKVTDPEVKEIIEGCIRQNKSERLSIRDLLNHAFFAE
DTGLRVE

Sequence after tag cleavage:

SMEAEMKAVATSPSGRFLKFDIELGRGAFKTVYKGLDTETWVEVAWCELQDRKLTAEQ
QRKFKEEAEMLKGLQHPNIVRFYDSWESILKGKKCIVLVTELMTSGTLKTYLKRFKVMKPK
KVLRSWCRQILKGLQFLHTRTPPIIHRDLKCDNIFITGPTGSVKIGDLGLATLMRTSFAKSV
IGTPEFMAPEMYEEHYDESVDVYAFGMCMLEMATSEYPYSECQNAAQIYRKVTSGIKPA
SFNKVTDPEVKEIIEGCIRQNKSERLSIRDLLNHAFFAEDTGLRVE

DNA Sequence:

ATGGCCACTGATTCAGGGGATCCAGCCAGCACAGAAGATTCTGAGAAACCTGATGGA
ATTTCAATTTGAAAACAGAGTTCCCCAGGTCGCTGCAACTTTGACAGTAGAAGCTAGAC
TAAAGGAGAAAAACAGTACCTTCTCTGCTTCTGGGGAAACTGTAGAAAGGAAGAGAT
TTTTCCGAAAGAGTGTTGAAATGACGGAAGATGACAAAGTTGCCGAATCATCCCCCA
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ACCATTCCAGCCAGGGATGAATATGCAGGCATTTCCAACCTTCATCAGTGCAGAATCCT
GCCACAATCCCTCCTGGTCCTAAATGA

Protein Expression in Insect cells

Medium: Insect XpressTM - from Lonza (Catalog no. BE12-730Q)

Antibiotics: Penicillin/Streptomycin at 0.1%(v/v) for preventing contamination

Procedure: *Sf9* cells grown to log phase at 2×10^6 /ml, and co-infected with 3 ml WNK3B-c005 P2 virus /L, cells incubated at 27°C, shaking at 100 rpm in Infors shakers(with 25mm throw), cells harvested 72h post infection.

Baculoviruses amplified in 250ml glass Erlenmeyer flasks, 50ml *Sf9* cells at 2×10^6 /ml supplemented with 2% v/v foetal bovine serum, harvested 66-72h post infection, with supernatant collected by centrifugation at 900g, 4°C. (the pellet can be used for small scale purification)

Protein Purification

Procedure: Cells were lysed by sonication, 0.125% polyethyleneimine added, and lysates were clarified by centrifugation at 50,000G. The supernatant was then applied to a Nickel-sepharose gravity column and washed and eluted with 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, and 10-300mM imidazole. Protein containing fractions were then treated with TEV protease overnight at 4 °C prior to gel filtration on an S200 gel filtration column using 50 mM HEPES pH 7.5, 150 mM NaCl, 1 mM TCEP as the running buffer. The protein was concentrated to 12.5 mg/ml using a spin concentrator.

Columns: Column 1: Ni²⁺ affinity; Column 2: S200 Gel Filtration

Concentration: 12.5 mg/ml

Mass-spec Verification: Intact mass correct. Mostly monophosphorylated with some unphosphorylated.

Structure Determination

Crystallization: Crystals were grown using the sitting drop vapor diffusion method at 4 °C.

Crystals were grown in 150 nl drops consisting of 1:2 mother liquor (22.5% PEG Smear Broad -- 0.1M cacodylate pH 5.5 -- 0.1M sodium/potassium tartrate -- 10% ethylene glycol) to protein

(12.5 mg/ml). The protein solution additionally contained 1mM AMP-PNP and 2 mM MgCl_2 , although these are not observed in the crystal. 25% Glycerol was added and the crystal was then mounted and flash frozen in liquid nitrogen.

Data Collection: Data was collected at beamline I04 at 100K.

Data Processing: Data was processed to a resolution of approximately 2.1 Å using XDS and either xia2 or autoPROC, or DIALS.