KCNAB2B

PDB:1ZSX

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:KCNAB2B-s001
Entry Clone Source: Origene
SGC Clone Accession:
Tag:(His 6 + TEV cleavage site): mgsshhhhhhssgrenlyfq*gh
Host:

Construct

Prelude:
Sequence:

mgsshhhhhhssgrenlyfqghMLQFYRNLGKSGLRVSCLGLGTWVTFGGQITDEMAEQLMTLAYDNGINLFDTAEVYAAGKAEVVL
GNIIKKKGWRRSSLVITTKIFWGGKAETERGLSRKHIIEGLKASLERLQLEYVDVVFANRPDPNTPMEETVRAMTHVINQGMAMYWG
TSRWSSMEIMEAYSVARQFNLTPPICEQAEYHMFQREKVEVQLPELFHKIGVGAMTWSPLACGIVSGKYDSGIPPYSRASLKGYQWL
KDKILSEEGRRQQAKLKELQAIAERLGCTLPQLAIAWCLRNEGVSSVLLGASNADQLMENIGAIQVLPKLSSSIIHEIDSILGNKPY

Vector:p11

Growth

Medium:
Antibiotics:
Procedure: The KCNAB2B construct was expressed in E. coli (BL21 DE3) in 1 L LB Broth in the presence of 100 Âµg/ml of ampicillin at 37 Â°C. Cells were induced at 1 mM IPTG as soon as the OD reached 0.8, and temperature was shifted to 18 Â°C, and culture was grown for 12 hrs. Cells were collected by centrifugation, and pellets were stored frozen (-20Â°C) until further use.

Purification

Buffers
Procedure
Column 1: HiTrap His

Buffers (adjusted to pH 7.5): Lysis buffer: 5mM Imidazole, 500mM NaCl, 50mM HEPES, 5%Glycerol. Wash buffer: 30mM Imidazole, 500mM NaCl, 50mM HEPES, 5%Glycerol.

Elution Buffer: 250mM Imidazole, 500mM NaCl, 5 0mM HEPES, 5% glycerol.

Procedure: Sample was loaded, washed with wash buffer and eluted in elution buffer. The collected peak was injected into size-exclusion chromatography system, and the main peak was selected for concentration using an Amicon Ultra device.

Column 2 : Superdex S200

Extraction

Buffers
Procedure
Pellets were resuspended in 25 mL lysis buffer including Protease inhibitor (complete, Roche), lysed by French Press, and the solution was centrifuged to obtain a clear supernatant (30 min, 17,000 rpm). Supernatants were processed in a 2 step chromatographic procedure using the Akta xpress (GE Healthcare) purification system.
Concentration:
Ligand
MassSpec:
Crystallization: Column 1: HiTrap His

Buffers (adjusted to pH 7.5): Lysis buffer: 5mM Imidazole, 500mM NaCl, 50mM HEPES, 5%Glycerol. Wash buffer: 30mM Imidazole, 500mM NaCl, 50mM HEPES, 5%Glycerol.

Elution Buffer: 250mM Imidazole, 500mM NaCl, 5 0mM HEPES, 5% glycerol.

Procedure: Sample was loaded, washed with wash buffer and eluted in elution buffer. The collected peak was injected into size-exclusion chromatography system, and the main peak was selected for concentration using an Amicon Ultra device.

Column 2 : Superdex S200
NMR Spectroscopy:
Data Collection:
Data Processing: