VRK3

PDB:2JII

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|31982929
Entry Clone Source: FivePrime
SGC Clone Accession:VRK3A-c016
Tag:mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*smTTSLEALPTGTVLTDKSGRQWKLKSFQTRDNQGILYEAAPTSTLTCDSGPQKQKFSLKLDAKD
GRLFNEQNFFQRAAKPLQVNKWKKLYSTPLLAIPTCMGFGVHQDKYRFLVLPSLGRSLQSALDVSPKHVLSERSVLQVACRLLDALE
FLHENEYVHGNVTAENIFVDPEDQSQVTLAGYGFAFRYCPSGKHVAYVEGSRSPHEGDLEFISMDLHKGCGPSRRSDLQSLGYCMLK
WLYGFLPWTNCLPNTEDIMKQKQKFVDKPGPFVGPCGHWIRPSETLQKYLKVVMALTYEEKPPYAMLRNNLEALLQDLRVSPYDPIG
LPMVP

Vector:pNIC28-Bsa4

Growth

Medium:LB
Antibiotics:
Procedure:1ml from a 10 ml overnight culture containing 50 µg/ml kanamycine and 35 µg/ml chloramphenicole was used to inoculate 1 liter of LB media containing the same concentration of antibiotics. Cultures were grown at 37°C until the OD600 reached ~0.3. After that the temperature was adjusted to 20°C. Expression was induced for 4 hours using 1mM IPTG at an OD600 of 0.8. The cells were collected by centrifugation and the pellet re-suspended in binding buffer and frozen.

Purification

Procedure
Column 1: Ni-affinity chromatography.
Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)

5 ml of 50% Ni-NTA slurry (Qiagen) was applied to a 1.5 x 10 cm gravity column. The column was equilibrated with 50 ml binding buffer. The lysate was applied to the column which was

subsequently washed with 50 ml wash buffer 1 and 2. The protein was eluted by gravity flow by applying 5 ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150mM, 250 mM); fractions were collected until essentially all protein was eluted. The eluted protein was analyzed by SDS - PAGE . DTT was added to the protein sample to a final concentration of 10mM.

The fractions eluted of the Ni-affinity chromatography were concentrated to about 4 mls using Centricon concentrators (10kDa cut off). The concentrated protein was applied to a Superdex S75 column equilibrated in SEC buffer at a flow rate of 0.8 ml/min. Eluted fractions were 95% pure as judged by SDS-PAGE.

Extraction

Procedure
Cell pellets were lysed by sonication. The lysate was centrifuged at 19,000 rpm for 60 minutes and the supernatant collected for purification.
Concentration: Centricon with a 10kDa cut off in SEC-buffer.
Ligand
MassSpec: The mass of the cleaved protein determined by ESI-MS-tof corresponded to the theoretical mass of 39850 Dalton calculated for the construct before TEV cleavage.
Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 11 mg/ml by mixing 75nl of the concentrated protein with 75nl of a well solution containing 0.1M HEPES pH 7.5; 2M Ammonium formate. The non TEV cleaved protein was used. Crystals appeared after a couple of days at 4°C.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected using the well solution supplemented with an additional 20% ethylene glycol and flash frozen in liquid nitrogen. Diffraction data were collected at the SLS beam line X10 (λ=1.0331 Å) to 2.0 Å resolution.
Data Processing: