STK10: Human Serine Threonine kinase 10 (STK10, LOK)

PDB:2J7T

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|5174701
Entry Clone Source:synthetic DNA
SGC Clone Accession:
Tag: mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3)

Construct

Prelude: The following mutations have been detected in that clone with respect to the reference sequence: V62A, E136V, G317E. The mutations have been confirmed by electron density and ESI-MS.
Sequence:

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smRKSREYEHVRRDLDPNEVWEIVGELGDGAFGKVYKAKNKETGALAAAKVIETKSEEELEDYIVEIEILATCDHPYIVKLLGAYYH
DGKLWIMIEFCPGGAVDAIMLELDRGLTEPQIQVVCRQMLEALNFLHSKRIIHRDLKAGNVLMTLEGDIRLADFGVSAKNLKTLQKR
DSFIGTPYWMAPEVVMCETMKDTPYDYKADIWSLGITLIEMAQIEPPHHELNPMRVLLKIAKSDPPTLLTPSKWSVEFRDFLKIALD
KNPETRPSAAQLLEHPFVSSITSNKALRELVAEAKAEVMEE
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Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:1ml from a 10 ml overnight culture (BL21 DE3) 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 resuspended in binding buffer and frozen. Binding buffer: 50mM HEPES pH 7.5; 300 mM NaCl; 20 mM imidazole.

Purification

Procedure
Column 1: Ni-affinity chromatography.
Procedure: 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. STK10 was eluted with 25 mls of elution buffer. The eluted protein was collected and analyzed by SDS-PAGE. DTT was added to the protein sample to a final concentration of 10mM. The N-terminal his 6 -tag was cleaved by incubating the protein overnight with TEV protease.

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

Protein concentration: Centricon with a 10kDa cut off in SEC-buffer

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:
Ligand
MassSpec:
Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 12.83 mg/ml containing 1 mM SU11274 ((3Z)-N-(3- Chlorophenyl)-3-((3,5-dimethyl -4-((4-methylpiperazin -1-yl)carbonyl) -1H-pyrrol-2-yl)methylene) -N-methyl-2-oxo -2,3-dihydro-1H-indole -5-sulfonamide) by mixing 100nl of the concentrated protein with 50nl of a well solution containing 45% PEG300, 0.24M CaAc, 0.1M Na cacodylate pH 6.5) Crystals appeared after a couple of days at 4°C.
NMR Spectroscopy:
Data Collection: Crystals were directly flash frozen in liquid nitrogen. Diffraction data were collected at the SLS beam line X10 (λ=0.999 Å) to 2.0Å
Data Processing: