STK16

PDB:2BUJ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|4505837
Entry Clone Source:TKC
SGC Clone Accession:
Tag:N-terminal histag with TEV-protease cleavage site (*): mgsshhhhhhssgrenlyfqgh*m
Host:E. coli BL21(DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssgrenlyfqghMVIIDNKHYLFIQKLGEGGFSYVDLVEGLHDGHFYALKRILCHEQQDREEAQREADMHRLFNHPN
ILRLVAYCLRERGAKHEAWLLLPFFKRGTLWNEIERLKDKGNFLTEDQILWLLLGICRGLEAIHAKGYAHRDLKPTNILLGDEGQPV
LMDLGSMNQACIHVEGSRQALTLQDWAAQRCTISYRAPELFSVQSHCVIDERTDVWSLGCVLYAMMFGEGPYDMVFQKGDSVALAVQ
NQLSIPQSPRHSSALWQLLNSMMTVDPHQRPHIPLLLSQLEALQPPAPGQHTTQIL

Vector:modified pET15b containing a TEV cleavage site

Growth

Medium:
Antibiotics:
Procedure:10 mL overnight cultures in LB, 100 µg/mL ampicillin were centrifuged, resuspended in fresh buffer, and used to inoculate 1 litre of LB medium containing 100 µg/mL ampicillin. Cultures were grown at 37°C until they reached an OD600 of 0.3 and then cooled to 18°C. Expression was induced for 4 hours using 1 mM IPTG at an OD600 of 0.8. The cells were collected by centrifugation, transferred to 50 mL tubes, resuspended in 30 mL binding buffer, and frozen. Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol.

Purification

Procedure
Column 1 : Ion exchange - Nucleic acid removal. DEAE cellulose (DE52, Whatmann), 10 g of resin in 2.5 x 20 cm column. The resin was hydrated in 2.5M NaCl , and then washed with 20 mL binding buffer prior to loading the sample. Procedure : Supernatant was applied at gravity flow, followed by a wash with 20 mL binding buffer. The column flow-through was collected.

Column 2: Ni-affinity. Ni-NTA (Qiagen), 5 mL of 50% slurry in 1.5 x 10 cm column, washed with binding buffer. Buffers: Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl , 5% Glycerol. Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl , 20 mM Imidazole , 5% glycerol. Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 50 to 250 mM Imidazole, 5% Glycerol. Procedure: The flowthrough from column 1 was loaded by gravity flow on the Ni-NTA column. The column was then washed with 3 x 10 mL wash buffer at gravity flow. The protein was eluted by gravity flow by applying 5 mL portions of elution buffer with increasing concentration of imidazole (50 mM , 100 mM , 250 mM); fractions were collected until essentially all protein was eluted. After elution DTT was added to a final concentration of 10 mM .

Enzymatic treatment : Dephosphorylation (lambda; - phosphatase): a GST fusion with the lambda phosphatase . TEV protease cleavage. Both treatments carried out simultaneously: protein solution contained 10 mM DTT and 0.05 mM MnCl 2 (higher MnCl 2 concentrations caused precipitation).

Column 3: Ion exchange Mono Q column. Buffers: A : 50 mM Hepes pH 7.5>. B : 50 mM Hepes pH 7.5, 1000 mM NaCl. Procedure: D ephosphorylated protein was applied to MonoQ in buffer A and eluted from the column by a linear gradient.

Column 4: SEC. Buffers: Fractions containing STK16 collected from ion exchange chromatography were concentrated and applied to a S75 column equilibrated in 50 mM Hepes pH 7.5, 100 m NaCl.

Extraction

Procedure
The frozen cells were thawed on ice and lysed using a high pressure cell disruptor. The lysate was centrifuged at 17000 RPM for 30 minutes. Supernatant was collected and binding buffer was added to 50 mL.
Concentration:
Ligand
MassSpec:
Crystallization: Column 1 : Ion exchange - Nucleic acid removal. DEAE cellulose (DE52, Whatmann), 10 g of resin in 2.5 x 20 cm column. The resin was hydrated in 2.5M NaCl , and then washed with 20 mL binding buffer prior to loading the sample. Procedure : Supernatant was applied at gravity flow, followed by a wash with 20 mL binding buffer. The column flow-through was collected.

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NMR Spectroscopy:

Data Collection:

Data Processing: