STARD5

PDB:2R55

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|45945558
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m)
Host:*E.coli* BL21(DE3) (Novagen)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*sMAAQMSEAVAEKMLQYRRDTAGWKICREGNGVSVSWRPSVEFPGNLYRGEGIVYGTLEEVWDCV
KPAVGGLRVKWDENVTGFEIIQSITDTLCVSRTSTPSAAMKLISPRDFVDLVLVKRYEDGTISSNATHVEHPLCPPKPGFVRGFNHP
CGCFCEPLPGEPTKTNLVTFFHTDLSGYLPQNVVDSFFPRSMTRFYANLQKAVKQFHE

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 20 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin at 30 ºC overnight. The overnight culture (20 ml) was used to inoculate 1.5 l TB supplemented with 16 g/l glycerol, 50 µg/ml kanamycin and approximately 100 µl BREOX FMT 30 anti-foam solution (Cognis Performance Chemicals UK Ltd). The culture was grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 ºC until OD600 reached ~2.2. The culture was down-tempered to 18 ºC over a period of 1.5 hours before target expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight and cells were harvested the following morning by centrifugation (3,500 x *g*, 10 min, 4 ºC). The resulting cell pellet (19.5 g wet cell weight) was resuspended in lysis buffer (3 ml/g cell pellet), supplemented with two tablets of Complete EDTA-free protease inhibitor (Roche Applied Science). The cell suspension was stored at -80 ºC.

Purification

Procedure

Column
IMAC: Ni-charged 1 ml HiTrap Chelating HP (GE Healthcare)
Gel filtration column: HiLoad 16/60 Superdex 75 Prep Grade (GE Healthcare)

Procedure
Purification of the protein was performed as a two step process on an ÄKTAxpress system (GE Healthcare). Prior to purification, columns were equilibrated with IMAC wash1 buffer and gel filtration buffer, respectively. The filtered lysate was loaded onto the Ni-charged HiTrap Chelating column and washed with IMAC wash1 buffer followed by IMAC wash2 buffer. Bound protein was eluted from the IMAC column with IMAC elution buffer and automatically loaded onto the gel filtration column. The chromatogram from gel filtration showed one major protein peak that consisted of highly pure target protein as shown by SDS-PAGE analysis. Fractions containing the target protein were pooled and fresh TCEP was added to a final concentration of 2 mM. The protein was subsequently concentrated using a Amicon Ultra-15 centrifugal filter device, 5,000 NMWL (Millipore) to 21.8 mg/ml in a volume of 1.0 ml. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in water and 2000 U Benzonase (Merck) were added. The cells were disrupted by sonicated (Vibra-Cell, Sonics) at 70% amplitude for 30 s effective time (pulsed 1s on, 1s off) followed by high-pressure homogenization (TC5-0612W-332 from Stansted fluid power LTD). The cell debris was removed by centrifugation (49,100 x *g*, 20 min, 4 ºC) and supernatant was decanted and filtered through a 0.45 µm flask filter.
Concentration:
Ligand
MassSpec:
Crystallization: The crystal was obtained by the sitting drop vapour diffusion method in a 96-well plate. 0.8 µl protein solution (21.8 mg/ml) was mixed with 0.4 µl of well solution consisting of 20 mM HEPES pH 7.0 and 10% PEG 6000. The plate was incubated at 4 ºC and crystals appeared after ~22 days. The crystal were quickly transferred to cryo solution and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data to 2.5 Å resolution was collected at ESRF beamline ID14-4. A single crystal was used to collect 360º oscillation range.
Data Processing: Data was processed with XDS in space group P65 (a=b=62.87 Å, c=214.93 Å). The structure was solved by molecular replacement using PDB entry 1JSS as model. Model building was done with COOT. PHENIX was used in the early refinement and REFMAC5 in the final refinement stages.