ITCH

PDB:3TUG
Entry Clone Accession:BC011571
Entry Clone Source:MGC:AU66-H3
SGC Clone Accession:SDC094-D06
Tag:N-terminal His6-tag, not removed
Host:BL21-V2R
Vector:pET28-MHL
Prelude:itch.483.862This construct has two serenpiditous mutations L564S, H760R relative to reference sequence NP_113671.3

Sequence:

mhhhhhhssgrenlyfqgYVRDFKAKVQYFRFWCQQLAMPQHIKITVTRKTLFEDSFQQIMSFSPQDLRRRLWVIFPGEEGLDYGGV
AREWFFLLSHEVSNPMYCLFEYAGKDNYCLQINPASYINPDHLKYFRFIGRFIAMALFHGKFIDTGFSLPFYKRILNKPVGLKDLES
IDPEFYNSLIWVKENNIEECDLEMYFSVDKEILGEIKSHDLKPNGGNILVTEENKEEYIRMVAEWRLSRGVEEQTQAFFEGFNEILP
QQYLQYFDAKELEVLLCGMQEIDLNDWQRHAIYRRYARTSKQIMWFWQFVKEIDNEKRMRLLQFVTGTCRLPVGGFADLMGSNGPQK
FCIEKVGKENWLPRSHTCFNRLDLPPYKSYEQLKEKLLFAIEETEGFGQE - Sequence verified by DNA sequencin
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Growth

Medium:LEX Bubbling. The target protein was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 2 L of Terrific Broth medium in the presence of 50 microg/mL kanamycin, 600 uLantifoam at 37 degree. When OD600 reached ~6.0, the temperature of the medium was lowered to 15 degree and the culutre was induced with 0.1 mM IPTG. The cells were allowed to grow overnight before harvested by centrifugation (12,227g 20min) and flash frozen in liquid nitrogen and stored at -80 degree.

Purification

Procedure: The lysate was centrifuged at 15,000 rpm for 45 minutes and the supernatants were loaded onto 3 mL Talon metal-affinity resin column (BD Biosciences) at 4 degree. The column was then washed 3 times with 15 mL washing buffer. Bound proteins were eluted using 6 mL elution buffer. The flow-through was collected and further purifed by a Superdex-200 gel filtraton column pre-equilibrated with gel filtration buffer. Fractions containing the target protein were pooled and concentrated

using Amicon Ultra-15 centrifugal filter (mwco 10 kDa). The purity of the preparation is tested by SDS-PAGE to be greater than 95%.

Extraction

Procedure: Frozen cells from 4L TB culture were thawed and resuspended in 120 mL extraction buffer, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 uL benzonase (Sigma Catalog # E1014, 250U/uL), and lysed using Microfluidizer (Microfluidics M110-EH) at 18,000 psi.

Concentration:11.7 mg/mL

Structure Determination

MassSpec:uncut version native protein expected 47429.3, measured 47456.1 (47506.7, 47634.7)
Crystallization: Crystal was initially obtained from SGC-I screen condition A07.Crystal used for structure refinement was grown in 30% PEG1500, 0.2M NaCl 0.1M HEPES pH 7.5 in sitting drop setup, using 0.8uL protein, 0.8uL well solution, 0.2uL 3M NDSB-195 (from Hampton Additive Screen Kit) against 0.1 mL reservoir buffer at room temperature. Crystals grow to moutable size in 2-3 days.No Cryo used