BIRC6

PDB:3CEG
Entry Clone Accession:gi|12585192
Entry Clone Source:birc6.AB033115.KAZ.KIAA1289hh15326s2.pBluescriptIISKplus
SGC Clone Accession: Birc6.4470.4792.136F10 (SDC136F10)
Tag:N-terminal: MHHHHHHSSGRENLYFQG
Host:BL21 (DE3)
Vector:pET28-MHL

Sequence (with tag):
mhhhhhhssgrenlyfqgANQEKKLGEYSKKAAMKPKPLSVLKSLEEKYVAVMKKLQFDTFEM
VSEDEDGKLGFKVNYHYMSQVKNANDANSAARARRLAQEAVTLSTSLPLSSSSSVFVRC
DEERLDIMKVLITGPADTPYANGCFEFDVYFPQDYPSSPPLVNLETTGGHSVRFNPNLYND
GKVCLSILNTWHGRPEEKWNPQTSSFLQVLVSVQSLILVAEPYFNEPGYERSRGTPSGTQS
SREYDGNIRQATVKWAMLEQIRNPSPCFKEVIHKHFYLKRVEIMAQCEEWIADIQQYSSD
KRVGRTMSHHAAALKRHTAQLREELLKLPCPEGLDPD

Growth

Medium:TB
Procedure: The protein was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37ºC to an OD600 of 7.5. Protein expression was induced 0.2 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15ºC. The culture was centrifuged (12,000 x g, 15 minutes) and cell pellets collected and stored at -80ºC. In order to obtain the selenomethionyl derivative of the birc6 ubc domain, the cells were grown in M9 medium supplemented with glycerol using a M9 SeMET High-Yield growth media kit package (MD045004-50L, Medicilon) according to manufacturerÂ□s instructuion and the protein was purified as below.

Purification

Procedure: The cleared lysate was loaded onto a 3 mL TALON metal-affinity resin column (BD Biosciences) at 4ºC. The column was washed with 10 ml Wash buffer A, 10 ml Wash buffer B, and 10 ml Wash buffer A. The protein was eluted with 6 ml Elution buffer. The His-tag was removed by overnight incubation of the protein at 4ºC with TEV protease.

The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with Gel Filtration buffer. Fractions containing protein (analyzed by SDS-PAGE) were pooled and concentrated by ultrafiltration using an Amicon Ultra centrifugal filter with 10 kD cutoff to a final concentration of 40 mg/ml.

The yields of the protein and its selenomethionyl derivatives were approx. 12 and 2 mg per liter of bacterial culture, respectively.

Extraction

Procedure: The cell pellet from a 2 L culture was resuspended in 50 ml Lysis buffer, lysed using a Microfluidizer at 18,000 PSI, and cleared by centrifugation at 40,000 x g for 30 min.
Concentration:20 mg/ml

Structure Determination

MassSpec: Mass-spectroscopy by LCMS showed pure product of correct molecular weight corresponding (within 0.012%) to the birc6 ubc domain selenomethionyl derivative with His-tag removed and all 8 methionine residues substituted with selenomethionine.
Crystallization: Crystals of the birc6 ubc domain selenomethionyl derivative were grown at 298 K using the hanging drop method by mixing 1 volume of 20 mg/ml protein with 1 volume of well solution consisting of 11% PEG 8K, 0.1 M glycine buffer, pH 10.0 and 1 mM DTT. The crystals were cryoprotected by immersion in the well solution supplemented with 25% (v/v) glycerol.