BRPF1

PDB:3MO8

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:51173720
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQG
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:
Sequence:

gEDSPLDALDLVWAKCRGYPSYPALIIDPKMPREGMFHHGVPIPVPPLEVLKLGEQMTQEAREHLYLVLFFDNKRTWQWLPRTKLVP
LGVNQDLDKEKMLEGRKSNIRKSVQIAYHRALQHRSKVQGEQS

Vector:pET28-MHL

Growth

Medium:TB
Antibiotics:
Procedure:BRPF1 was expressed in E.coli BL21 (DE3) codon plus in Terrific Broth medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37oC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM and incubated overnight at 15oC.

Purification

Procedure
The lysate was loaded onto 5 ml HiTrap column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM HEPES, pH 7.4, containing 500 mM NaCl and 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM HEPES pH 7.4, 500 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was dialyzed against buffer containing 20 mM HEPES, pH 7.4, 500 mM NaCl and 5% glyceral. TEV protease was added to combined fractions containing BRPF1. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM PIPES, pH 6.5, and eluted with linear gradient of NaCl up to 500 mM

concentration (20CV). Purification yield was 12 mg of the protein per 1L of culture.
Enzymatic treatment: Thrombin

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80°C. For the purification the cell paste was thawed and resuspended in lysis buffer (50 mM HEPES, pH 7.4, 500 M NaCl, 2 mM β-mercaptoethanol, 5% glycerol, 0.1% CHAPS) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:48 mg/ml
Ligand
MassSpec: Expected MW is 15052.51 Da, measured mass is 15052.9917 Da.
Crystallization: Purified BRPF1 was complexed with H3K36me3 peptide **(PATGGVK(me)3KPHRY**, protein: peptide molar ratio at 1:5) and crystallized using sitting drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution (10 mg/mL) with 1 µl of the reservoir solution containing 3.5 M sodium formate, 0.1 M Tris-HCl, pH 8.5.
NMR Spectroscopy:
Data Collection:
Data Processing: