TRIM54

PDB:3Q1D

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:trim54.LIFESEQ4012106.OBS.IHS1382-8648091.pINCY 95
Entry Clone Source: Open Biosystems
SGC Clone Accession:trim54.0110-0171.192G05 (SDC192G05)
Tag:N-terminal: MHHHHHHSSGRENLYFQG
Host: Competent BL21 (DE3) cells (Invitrogen, C6000-03)

Construct

Prelude:
Sequence:

MHHHHHHSSGRENLYFQGQESSRPLHSKAEQHLMCEEHEEEKINIYCLSCEVPTCSLCKVFGAHKDCEVAPLPTIYKRQK

Vector:pET28MHL vector (GenBank, EF456735)

Growth

Medium:TB (Sigma, T0918) supplemented with 150 mM glycerol, 100 µM kanamycin, and 600 µl antifoam 204 (Sigma A-8311)
Antibiotics:
Procedure: Competent BL21 (DE3) cells (Invitrogen, C6000-03) were transformed and grown using the LEX system (HarbingerBiotech) at 37 °C in 1L bottles (VWR, 89000-242) containing 900 ml of growth medium. When the OD_{600} reached a value of about 6.0, the temperature was reduced to 19 °C, and one hour later the culture was induced with 500 µM IPTG (BioShop, IPT001) and incubated overnight (16 hours) at 19 °C.

Purification

Procedure
Protein was purified using the Streamline purification system (1). Briefly, bacterial cultures were directly microfluidized with the outflow connected to a novel column assembly, containing IMAC beads; a few hundred milliliters of regular buffer was also fluidized to wash the beads, and columns were eluted with imidazole by gravity. To each 1L culture bottle the following was sequentially added: NaOH (final pH ~7.5), imidazole (final 8mM), and BME (final 1 mM). Each bottle was fluidized through a Microfluidizer (model M110-EH with H10Z ceramic chamber performed at about 15,000 psi) with the outflow directly connected to a special nozzled-column containing 3 ml of settled HisLink (Promega, V8821). Approximately 200 ml fluidizer buffer was also fluidized to wash the beads. Columns were gravity-washed with 20 mL wash buffer, and protein was gravity-eluted with 8.0 ml elution buffer. Eluate was dialyzed overnight at 4 oC against about 100 volumes of dialyses buffer. Samples were concentrated using a 3 KDa MW cut-off concentrator (Millipore, UFC900524) at 3500 xg to a final value of 0.2 mM (~14 mg/mL). Protein yield was 5 mg per liter of bacterial culture.

1. Alenkin D, Yermekbayeva L, Mujib S, Vesterberg A, Newman E, Yamazaki K, Cossar D, Dhe-Paganon S. A centrifugation-free high-throughput protein purification system using in-line microfluidization. *Protein Expr Purif*. 2011.

Extraction

Procedure

Concentration:~14 mg/mL
Ligand
MassSpec:MW = 9339.55 g/mol
Crystallization: Crystals were grown at 18 oC in hanging drop plates (Hampton, HR3-170) by mixing equal volumes of protein (14 mg/ml) and Crystallization Buffer (1.4 M NaCit, 0.1 M Hepes pH 7.5, 5% MPD). Immediately prior to setting-up crystallization plates, trypsin was added to the protein sample to a final concentration of 5.7e-7 M (0.57 micromolar) and protein concentration of 1.5e-3 M (1.5 millimolar). Suitable crystals were dunked directly into liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: