USP15

PDB:3PPA
Entry Clone Accession:BC125123.MGC.CM34-A7.pCR-BluntII-TOPO
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:usp15.0006x0223.206G08 (SDC206G08)
Tag:N-terminal: MGSSHHHHHHSSGLVPRG
Host: Competent BL21 (DE3)-V2R cells (Invitrogen, C6000-03???)
Vector:pET28a-LIC vector (GenBank EF442785)

Sequence:

MGSSHHHHHHSSGLVPRGSAADLDTQRSDIATLLKTSLRKGDTWYLVDSRWFKQWKKYVGFDSWDKYQMGDQNVYPGPIDNSGLLKD
GDAQSLKEHLIDELDYILLPTEGWNKLVSWYTLMEGQEPIARKVVEQGMFVKHCKVEVYLTELKLCENGNMNNVVTRRFSKADTIDT
IEKEIRKIFSIPDEKETRLWNKYMSNTFEPLNKPDSTIQDAGLYQGQVLVIEQKNEDGTWPRG

Growth

Medium:TB (Sigma, T0918) supplemented with 150 mM glycerol, 100 µM Kanamycin and 600 µl antifoam 204 (Sigma A-8311)

Procedure: Competent BL21 (DE3)-V2R cells (Invitrogen, C6000-03???) were transformed and grown using the LEX system (HarbingerBiotech) at 37 °C in 1 L bottles (VWR, 89000-242) containing 800 ml of growth medium. When the OD_{600} reached a value of about 6.0, the temperature was reduced to 15 °C, and one hour later the culture was induced with 100 µM IPTG (BioShop, IPT001) and incubated overnight (16 hours) at 15 °C.

Purification

Procedure: A volume of 1.0 mL settled TALON resin per 60 mL lysate (Clontech) was rocked with unclarified lysate for 60 minutes at 4 °C, washed with 45 mL of cold Wash Buffer, spun at 1000 xg for 5 minutes, and transferred to a column. After additional washing (50 column volumes), protein was eluted with 5 mL (per mL of settled TALON resin) of Elution Buffer and dialyzed overnight at 4 oC against 200 volumes of Dialyses Buffer. The protein sample was concentrated using a 10 KDa MW cut-off concentrator (Millipore, UFC900524) at 3500 xg to a final value of 1mM (~40 mg/mL). Protein yield was 10 mg per liter of bacterial culture.

Extraction

Procedure: After resuspension in 30 mL per liter bacterial culture of Lysis buffer, cells were lysed using a microfluidizer (Microfluidics, M110-EH) at 18,000 psi.
Concentration:~40 mg/mL

Structure Determination

MassSpec:MW =27,378 g/mol

Crystallization: Crystals were grown at 18 oC using the sitting drop method in Intelliplates (Art-Robbins Instruments) by mixing equal volumes of protein (23 mg/ml) and Crystallization Buffer (0.5M NH_4SO_4, 1M $LiSO_4$, 0.1M Citrate pH5.6), with the addition of 1 uM Dispase (Sigma, D4818-2mg). Suitable crystals were cryoprotected by immersion in well solution supplemented with 50:50 paratone:mineral oil prior to dunking and storage in liquid nitrogen.