USP7

PDB:4PYZ
Entry Clone Accession:NM_003470
Entry Clone Source: Origene, SGC:02-E6
SGC Clone Accession:SDC229B03
Tag:N-terminal His6-tag, not removed
Host:BL21(DE3)-V2R
Vector:pET28a-LIC
Prelude:USP7.0537-0793; Uniprot:Q93009

Sequence:

GSSHHHHHHSSGLVPRGSPQQLVERLQEEKRIEAQKRKERQEAHLYMQVQIVAEDQFCGHQGNDMYDEEKVKYTVFKVLKNSSLAEF
VQSLSQTMGFPQDQIRLWPMQARSNGTKRPAMLDNEADGNKTMIELSDNENPWTIFLETVDPELAASGATLPKFDKDHDVMLFLKMY
DPKTRSLNYCGHIYTPISCKIRDLLPVMCDRAGFIQDTSLILYEEVKPNLTERIQDYDVSLDKALDELMDGDIIVFQKDDPENDNSE
LPTAKEYFRDLYHR

Growth

Procedure: LEX Bubbling. The target protein was expressed in E. coli by inoculating 25 mL of overnight culture grown in Luria-Bertani medium into a 2 L Terrific Broth medium in the presence of 50 ug/mL kanamycin at 37 degree celsius. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 degree celsius and the culture was induced with 1 mM IPTG. The cells were allowed to grow overnight before harvested by centrifugation (7,000 rpm 10min) and flash frozen in liquid nitrogen before storage at -80 degree celsius.

Purification

Procedure: The lysate was centrifuged at 16,000 rpm for 60 minutes and the supernatant was incubated with 3 mL of Ni-NTA agarose resin, with constant rotation, for 1h. The bound protein was then spun down at 1,500 rpm for 5 minutes at 4 degree celsius before loading into an open column. The bound protein was then washed with a total of 30 mL of wash buffer, and eluted with 30 ml of elution buffer. The eluate was purified with gel filtration and the fractions containing the target protein were pooled and concentrated using Amicon Ultra-15 centrifugal filter (mwco 10 kDa). The purity of the preparation was assessed by SDS-PAGE and estimated to be >95% pure

Extraction

Procedure: 2L cell pellet was resuspended in a total volume of 200 ml lysis buffer and the cells were sonicated at 100W, with a 10s on and off pulse, for 10 minutes

Concentration: 14.0 mg/mL

Structural Determination

Crystallization:RW-A09 Optimization

Purified protein at 14mg/mL was mixed with a UHRF1 peptide (Ac-GKGKWKRKSAGGGPS-NH2,GenScript 95% purifity) at 1:5 molarity ratio, and sitting drop crystallization was setup. Initial crystal hit wasseen in Red Wings Screen condition A09. Crystal used for data collection wasgrown in hanging drop containing 1.5 uL protein/peptide mix and 1.5uL 15% PEG4000, 0.2 M NH4Ac, and 0.1 M NaCitrate at pH 5.6, 18°C.

The final crystal does not contain the UHRF1 peptide