TP53I3A: Human p53 inducible oxidoreductase

PDB:2J8Z

Revision

Revision Type:created
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Entry Clone Accession:BC000474
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Tag:
Host:E. coli BL21(DE3)-R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmLAVHFD KPGGPENLYVKEVAKPSPGEGEVLLKVAA SALNRADLMQRQGQYDPPPGASNILGL
EA SGHVAELGPGCQGHWKIGDTAMALLPGGG QAQYVTVPEGLLMPIPEGLTLTQAAAIPE AWLTAFQLLHLVGNVQAGDYVLIH
AGLSG VGTAAIQLTRMAGAIPLVTAGSQKKLQMA EKLGAAAGFNYKKEDFSEATLKFTKGAGV NLILDCIGGSYWEKNVNCLAL
DGRWVLYG LMGGGDINGPLFSKLLFKRGSLITSLLRS RDNKYKQMLVNAFTEQILPHFSTEGPQRL LPVLDRIYPVTEIQEAHK
YMEANKNIGKI VLELPQ

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Medium: TB + 50 µg/ml Kanamycin + 34 µg/ml chloramp. 1 liter TB in 2.5-L baffled flasks were inoculated with 10 ml overnight culture and grown at 37°C. The protein expression was induced with 1.0 mM IPTG at OD600 = 5.2 for 4 h at 25°C . The cells were collected by centrifugation and frozen at -80°C.

Purification

Procedure
Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences)
Procedure: The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected.

Column 2 : Gelfiltration, Hiload 16/60 Superdex 200 prep grade, 120 ml (GE/ Amersham Biosciences)
Procedure: The eluted fractions from the Ni-affinity Histrap column were loaded on the gel filtration column at 1.0 ml/min. Eluted proteins were collected in 2 ml fractions.

Concentration: The protein was concentrated in Amicon (10 K) to 15.1 mg/ml and the protein concentration determined spectrophotometrically using the predicted molar extinction coefficient 39390(M-1cm-1).

Extraction

Procedure

Concentration:
Ligand
MassSpec: The mass determined for TP53I3Ap003 was 38089 Da, in agreement with the predicted mass for the his-tagged protein.
Crystallization: Crystals were grown by vapor diffusion at 20°C. A sitting drop consisting of 300 nl protein (15.1 mg/ml) and 300 nl well solution was equilibrated against well solution containing 0.22 M Li2SO4, 0.1 M NaAc, 45% PEG300.
NMR Spectroscopy:
Data Collection: Resolution: 2.5 Å; X-ray source: Synchrontron SLS-X10.
Data Processing: