RRM2 - Human ribonucleotide reductase, R2 subunit

PDB:2UW2

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC001886
Entry Clone Source:
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

```
mhhhhhhssgvdlgtenlyfqsmTKAAAPGVEDEPLLRENPRRFVIFPIEYHDIWQMYKKAEASFWTAEEVDLSKDIQHWESLKPEE
RYFISHVLAFFAASDGIVNENLVERFSQEVQITEARCFYGFQIAMENIHSEMYSLLIDTYIKDPKEREFLFNAIETMPCVKKKADWA
LRWIGDKEATYGERVVAFAAVEGIFFSGSFASIFWLKKRGPMPGLTFSNELISRDEGLHCDFACLMFKHLVHKPSEERVREIIINAV
RIEQEFLTEALPVKLIGMNCTLMKQYIEFVADRLMLELGFSKVFRVENPFDFMENISLEGKTNFFEKRVGEYQRMGVMSSPTENSFT
LDADF
```

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure:BL21 (DE3) cells from glycerol stocks were grown in 20 ml Terrific Broth media supplemented with 8 g/L glycerol and 100 µg/ml kanamycin at 30 ºC over night. The following morning, 20 ml of the over night culture inoculated 1500 ml of Terrific Broth media supplemented with 50 µg/ml kanamycin, 8 g/L glycerol and approximately 50 µl BREOX (antifoam) in glass flasks in the Large Scale Expression System (LEX). Cells were grown at 37 degC until OD600 nm of 2.9 and were down-tempered to 18 ºC for one hour in water bath. Expression of target protein was induced by addition of IPTG to a oncentration of 0.5 mM. Protein expression was allowed to continue over night at 18 ºC.

Purification

Procedure
Columns: HiTrap Chelating 1 ml (IMAC); HiLoadÂ□ 16/60 Superdex 200 Prep Grade (Gel filtration)

Procedure:Purification was conducted automatically on an ÄKTA xpress system operated by UNICORN software at a flow of 0.8 ml/min. Prior to purification, columns were equilibrated with IMAC Bind/Wash1 Buffer (HiTrap Chelating), Gel filtration buffer (Superdex 200). The protein sample was loaded on the HisTrap HP column that was washed with IMAC Bind/Wash1 Buffer followed by IMAC Wash2 Buffer. Bound protein was eluted from the IMAC columns with IMAC Elution Buffer and loaded in the Gel filtration column. The chromatogram from Gel filtration showed one protein peaks that migrated as a RRM2A dimer. SDS-PAGE analysis showed that the peak contained RRM2A protein of high purity. Fractions corresponding to this peak were pooled and TCEP was added to a final concentration of 2 mM. The protein was concentrated to 39.4 mg/ml and stored at -80 degC.

Extraction

Procedure
Cells were harvested by centrifugation and pellets were resuspended in 50mM Sodium-Fosfate pH 7.5, 500mM NaCl, 10% glycerol supplemented with one tablet Complete EDTA-free protease inhibitor tablet and frozen at -80 ºC. The frozen cells were briefly thawed in warm water and 2000 U of Benzonase was added. Cells were disrupted by High Pressure Homogenization run twice at 10 000 PSI and samples were centrifuged for 20 minutes at 40000×g. The soluble fraction was filtered through 0.45 µm and subjected to further purification on the ÄKTAxpress.
Concentration:
Ligand
MassSpec:
Crystallization: The protein was crystallized by letting 0.1 µl of protein (mg/ml) and 0.1 µl of well solution equilibrate against a well solution containing 11% peg10K, 0.1 M Bis Tris pH5.5, 0.12M Ammonium acetate. The structure was solved using molecular replacement with the mouse R2 structure as template (1XSM)
NMR Spectroscopy:
Data Collection:
Data Processing: