NQO2

PDB:1ZX1

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NQO2A-p002
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: mgsshhhhhhssgrenlyfq*gh(m)
Host:BL21(DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssgrenlyfqghMAGKKVLIVYAHQEPKSFNGSLKNVAVDELSRQGCTVTVSDLYAMNFEPRATDKDITGTLSNPEV
FNYGVETHEAYKQRSLASDITDEQKKVREADLVIFQFPLYWFSVPAILKGWMDRVLCQGFAFDIPGFYDSGLLQGKLALLSVTTGGT
AEMYTKTGVNGDSRYFLWPLQHGTLHFCGFKVLAPQISFAPEIASEEERKGMVAAWSQRLQTIWKEEPIPCTAHWHFGQ

Vector:p11

Growth

Medium:
Antibiotics:
Procedure: The cells were grown at 37°C in 1 L Terrific broth (TB) containing 50 µg/mL ampicillin until reaching OD595 of 0.6. Then the temperature was shifted to 30°C and 0.3 mM IPTG was added to induce the expression. Incubation continued at 30°C for 15 hours before the cells were harvested by centrifugation.

Purification

Procedure
Column 1 : Ni-NTA batch column (Qiagen), 5 mL of 50% slurry in 1.5 x 10 cm column.

Buffers: Washing buffer: 500 mM NaCl, 5% Glycerol, 50 mM Tris-HCl pH 7.5, 30 mM Imidazole, 2 mM TCEP; Elution buffer: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 250 mM Imidazole, 2mM TCEP.

Procedure: The supernatant was loaded to the Ni-NTA column and equilibrated with the extraction buffer. The column was then washed with 100 mL washing buffer; NQO2 was eluted in 10 mL elution buffer.

Column 2 : Hi Load 16/60 Superdex 200

GF buffer: 100 mM NaCl, 5% Glycerol, 10 mM HEPES pH 7.5, 2mM TCEP

Procedure: Eluted fraction was loaded to the GF column to change the buffer and to achieve homogenous NQO2 for crystallization. Protein was concentrated using a 10000 MW cutoff Amicon Ultra concentration device.

Extraction

Procedure
Extraction buffer: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 5 mM Imidazole, 2 mM TCEP. Cell pellets from 1 liter were resuspended in 50 mL extraction buffer and then lysed by French Press. The lysate was centrifuged at 18,000 RPM for 1 hour. The cleared lysate was loaded to the Ni-NTA column.
Concentration:
Ligand
MassSpec:
Crystallization: Purified NQO2 was concentrated to 12.6 mg/mL and distributed into 50 µL aliquots before being flash frozen at -80°C. Crystals were grown using the hanging-drop vapour diffusion technique with 2 µL drops. Before set up of the plate, NQO2A was mixed with 50 mM CB1954, 20 µM FAD, and then mixed in a 1:1 ratio with reservoir solution (100mM Na-HEPES pH7.0, 2.0 M ammonium sulphate, 5 mM DTT).
NMR Spectroscopy:
Data Collection:
Data Processing: