

MGC4172

PDB:1XG5

Entry Clone Accession:gi:14598960

Entry Clone Source:Synthetic

Tag:mgssshhhhhssgrenlyfq*gh (N terminus). gs (C terminus). TEV protease cleavable at *

Host:BL21(DE3)

Construct

Sequence:

MARPGMERWRDRLALVTGASGGIGA AVARALVQQGLKVVG CARTVGNIEELAAECKSAGYPGTLIPYRC DLSNEEDILSMFSAIRSQ
HSGVDICINNAGLARPD TLLSGSTSGWKDMFN VNLALSICTREAYQSMKERNVDDGHIININSM SGHRVLP LSVTHFY SATKYAVT
ALTEGLRQELREAQTHIRATCISPGVVETQFAFKLHDKDPEKAAATYEQMKCLKPEDVAEAVIYVLSTPAHIQIGDIQMRPT

Vector:p11

Growth

Purification

Procedure

Procedure based on gravity feed chromatography. Sample applied to a 10 mL DE-52 column and washed through with 20 mL binding buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole. (WB) 50 mM Tris-HCl pH 7.5, 500 mM NaCl, 5% Glycerol, 30 mM Imidazole). The flow through was applied to a 2 mL Ni-NTA column, the Ni-NTA column was washed with 200 mL of WB and eluted with elution buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole) in 2 mL aliquots. Monitored the eluate for protein using Coomassie Blue Plus Protein Assay reagent. TEV clean up: The TEV cleaved protein was applied to a 1 mL Ni-NTA column and washed through with binding buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole). The flow through being collected. The eluate from the column was monitored and when all the unbound protein had flowed through. The column was eluted with elution buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole) into a fresh container.

Extraction

Procedure

50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole, PMSF to 1 mM added. Cell pellet was resuspended in the above buffer then frozen, the thawed resuspension was lysed using Avestin C-5 microfluidizer, 4 passes. Lysate spun in a JA-17 rotor at 17,000 rpm.

Concentration:49 mg/mL in 10 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 0.5 mM TCEP.

MassSpec:Expected mass 30425.48, observed mass 30426.

Crystallization: Protein concentration 25 mg/mL + NADPH at 5 mM. Crystallization solution: 100 mM Bis-Tris, pH 5.5, 200 mM Ammonium Acetate, 2% Glycerol (total 7%), 20% PEG 3350, 0.5 mM TCEP.