17β-HSD4

PDB:1ZBQ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:4504505
Entry Clone Source:synthetic
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: mgsshhhhhhssgrenlyfqghm. C-terminal: gs
Host:E.coli BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssgrenlyfqghMGSPLRFDGRVVLVTGAGAGLGRAYALAFAERGALVVVNDLGGDFKGVGKGSLAADKVVEEIRRR
GGKAVANYDSVEEGEKVVKTALDAFGRIDVVVNNAGILRDRSFARISDEDWDIIHRVHLRGSFQVTRAAWEHMKKQKYGRIIMTSSA
SGIYGNFGQANYSAAKLGLLGLANSLAIEGRKSNIHCNTIAPNAGSRMTQTVMPEDLVEALKPEYVAPLVLWLCHESCEENGGLFEV
GAGWIGKLRWERTLGAIVRQKNHPMTPEAVKANWKKICDFENASKPQSIQESTGSIIEVLSKIDSgs

Vector:pET-11 derivative

Growth

Medium:
Antibiotics:
Procedure: Medium: LB with 100 mg/L ampicillin. 1 L LB in 2.5 L baffled flasks was inoculated with an overnight culture. The culture was grown at 37°C to OD=0.6-0.7. 1 mM IPTG was then added and incubation continued over a period of 4-5hours at 37°C. The cells were then collected by centrifugation and frozen at -20°C.

Purification

Procedure

Extraction

Procedure

Extraction buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole, 1 mM PMSF, 0.5mM TCEP. Extraction buffer was added to frozen cells and the cells were resuspended. The cells were disrupted by sonication.

Concentration:

Ligand

MassSpec:

Crystallization: Hampton I screen condition 17: 0.2M lithium sulphate monohydrate, 0.1M TRIS hydrochloride pH 8.5, 30% w/v polyethylene glycol 4000. Vapour Diffusion, sitting Drop at temperature 293K

NMR Spectroscopy:

Data Collection:

Data Processing: