SPHK1

PDB:4V24

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Mammalian Gene Collection (IMAGE consortium clone ID 4871343).
SGC Clone Accession:
Tag:N-terminal, TEV protease cleavable hexahistidine tag
Host:

Construct

Prelude:
Sequence:

SMEPRVEVMDPAGGPRGVLPRPCRVLVLLNPRGGKGKALQLFRSHVQPLLAEAEISFTLMLTERRNHARELVRSEELGRWDALVVMS
GDGLMHEVVNGLMERPDWETAIQKPLCSLPAGSGNALAASLNHYAGYEQVTNEDLLTNCTLLLCRRLLSPMNLLSLHTASGLRLFSV
LSLAWGFIADVDLESEKYRRLGEMRFTLGTFLRLAALRTYRGRLAYLPVGRVGSKTPASPVVVQQGPVDAHLVPLEEPVPSHWTVVP
DEDFVLVLALLHSHLGSEMFAAPMGRCAAGVMHLFYVRAGVSRAMLLRLFLAMEKGRHMEYECPYLVYVPVVAFRLEPKDGKGVFAV
DGELMVSEAVQGQVHPNYFWMVS

Glu81 - Ser449 of SPHK1B isoform 2
The N-terminal residues,SM, derive from the vector.
Vector:pFB-LIC-Bse

Growth

Medium:
Antibiotics:
Procedure:

Purification

Buffers
Procedure
Cell Lysis: The resuspended cell pellet was thawed and lysed by sonication on ice. PEI (polyethyleneimine) was added to a final concentration of 0.15 %. The cell debris and precipitated DNA were spun down.Lysis Buffer:50 mM Tris pH 7.8, 200 mM NaCl, 20 mM Imidazole, 0.5 mM TCEP, Sigma protease inhibitor cocktail. Purification: Column 1: 5 ml of Ni-Sepharose in a 2.5 cm diameter gravity flow column. Column 1 Buffers: Binding Buffer: 50 mM Tris pH 7.8, 200 mM NaCl, 20 mM Imidazole, 0.5 mM TCEP, Sigma protease inhibitor cocktail

Wash Buffer 1:As Binding Buffer except 40 mM imidazole and 1M NaCl.Wash Buffer 2:As Binding Buffer except 60 mM imidazole.Elution Buffer:As Binding Buffer except 250 mM imidazole. Column 1 Procedure:The clarified supernatant was passed through the column. The column was washed with 100 mL of Binding Buffer, 50 mL each of Wash Buffer 1 and Wash Buffer 2, and 25 mL of Elution Buffer was passed through to elute the protein. Column 2 Procedure: GF Buffer:20 mM Tris pH 7.8, 200 mM NaCl, 0.5 mM TCEP, TEV protease was added and the sample dialysed overnight against GF Buffer.The sample was passed through 1 mL of Ni-Sepharose (pre-equilibrated into GF Buffer) followed by 5 ml of additional GF Buffer. The resin was eluted with 5 ml of GF Buffer containing 10 mM, 20 mM, 40 mM and then 250 mM imidazole. Column 3 Procedure: The flow-through and 10 mM imidazole fraction from Column 2 were combined and concentrated to 5 mL volume. The sample was injected onto an S200 16/60 GF Column (GE Healthcare).

Extraction

Buffers
Procedure
Expression: The protein was expressed using baculovirus infected Sf9 cells. Cells were infected at a density of 2,000,000 cells/ml for 48h. Cell harvest:Cells were spun at 800x g for 20 mins and the pellets resuspended in Lysis Buffer and then frozen at -20°C.
Concentration: Compound PF-543 and ADP were added to the pooled fractions from gel filtration and the sample left overnight. The sample was concentrated to 10 mg/ml (measured by 280 nm absorbance).
Ligand
MassSpec: Mass Spec Characterisation: Expected Observed41120 41120.1
Crystallization: Crystals grew from a mixture of 100 nL SPHK1 protein (10 mg/ml with 1 mM PF-543 and 1 mM ADP), and 50 nL of a well solution containing 37.5% MPD, 0. 1 M BisTris pH 5.5 and 0.1 M ammonium acetate.
NMR Spectroscopy:
Data Collection:
Data Processing: