Kinase domain of Toxoplasma specific rhoptry antigen ROP8

PDB:3BYV

Revision

Revision Type:created
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Entry Clone Accession:33.m00005
Entry Clone Source:
SGC Clone Accession:33.m00005:s:P210-Y568:B6
Tag:mgsshhhhhhssgrenlyfqg
Host:BL21-(DE3)-Ros-Ox

Construct

Prelude:no exact match in NCBI blast - use data from www.ToxoDB.org
Sequence:

PGDVVIEELFNRIPQANVRTTSEYMQSAADSLVSTSLWNTGQPFRVESELGERPRTLVRGTVLGQEDPYAYLEATDQETGESFEVHV
PYFTERPPSNAIKQMKEEVLRLRLLRGIKNQKQAKVHLRFIFPFDLVKDPQKKKMIRVRLDERDMWVLSRFFLYPRMQSNLQTFGEV
LLSHSSTHKSLVHHARLQLTLQVIRLLASLHHYGLVHTYLRPVDIVLDQRGGVFLTGFEHLVRDGARVVSSVSRGFEPPELEARRAT
ISYHRDRRTLMTFSFDAWALGLVIYWIWCADLPITKDAALGGSEWIFRSCKNIPQPVRALLEGFLRYPKEDRLLPLQAMETPEYEQL
RTELSAALPLY

Vector:p15-tev-lic

Growth

Medium:TB
Antibiotics:
Procedure: The 10x M9 solution contained 60g Na2HPO4, 30g KH2PO4, 10g NH4Cl and 5g NaCl per litter and the solution was autoclaved. The Â□Se-MetÂ□ medium was prepared by combination of 100mL 10x M9, 1 mL 1M MgSO4, 10 mL 40% glucose, 100 µL 0.5% thiamine, 300 µL 12.5 mg/mL FeSO4, 300 µL 0.166% biotin, 1 mL 100mg/mL Ampicillin, 0.5 mL 100mg/mL kanamycin. The final volume was adjusted to 1L using sterile water. The amino acid mixture was prepared fresh before each use by mixing 100 mg L-lysine, 100 mg L-phenylalanine, 100mg L-threonine, 50mg L-isoleucine, 50mg L-valine, 50mg L-leucine and 50mg L-selenomethionine in a total volume of 10 ml. After the amino acid were completely dissolved, the solution was filter-sterilized.

Purification

Procedure

The cleared lysate was loaded onto a 1.0-2.5 mL Ni-NTA (Qiagen) column (pre-equilibrated with Binding Buffer) at approximately 1.5-2.0 mL/min. The Ni-NTA column was then washed with 150 mL of Wash Buffer at 2-2.5 mL/min. After washing, the protein was eluted with Elution Buffer. The eluted sample was applied to a Sephadex S200 26/60 gel filtration column pre-equilibrated with Gelfiltration Buffer. The fractions corresponding to the eluted protein peak were collected and further concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The protein sample identity and purity were evalulated by mass spectroscopy and SDS-PAGE gel. The concentrated protein was stored at 4 degC. For long term storage, the protein was flash frozen and stored at -80 degC.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 4 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at -80 oC were thawed overnight at 4 °C on the day before purification. Prior to sonication, each pellet from 1 L of culture was pretreated with 0.5 % CHAPS and 500 units of benzonase for 40 minutes at room temperature. After 10 minutes sonication, the cell lysate was centrifuged using a Beckman JLA-16.250 rotor at 15,500 rpms for 45 minutes at 4 oC.
Concentration:
Ligand
MassSpec:
Crystallization: The uncut protein was set up in sitting drop vapour diffsion crystallization trial. Diffracting crystals were obtained in 23% PEG3350, 0.2 M MgNO3, 0.1 M tris at pH 8.4,with 0.3 microL protein at 13.4 mg/mL mixed with 0.3 microL buffer.
NMR Spectroscopy:
Data Collection:
Data Processing: