RGS1

PDB:2BV1

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:RGS 1A-s001
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag
Host:BL-21(DE3)R3

Construct

Prelude:
Sequence:

SMSGMKSSKSKDVLSAAEVMQWSQSLEKLLANQTGQNVFGSFLKSEFSEENIEFWLACEDYKKTESDLLPCKAEEIYKAFVHSDAAK
QINIDFRTRESTAKKIKAPTPTCFDEAQKVIYTLMEKDSYPRFLKSDIYLNLLNDLQA

Vector:pLIC-SGC

Growth

Medium:
Antibiotics:
Procedure:

Purification

Procedure
Column 1: Low pressure chromatography using Bio-Rad Econo column (2.5 cm x 13 cm).

Buffers: Wash Buffer I (WBI): 50 mM Hepes pH 8.0, 300 mM NaCl, 5 % glycerol, 10 mM imidazole pH 8.0; Wash Buffer II (WBII): 50 mM Tris pH 8.0, 300 mM NaCl, 5 % glycerol, 30 mM imidazole pH 8.0; Elute Buffer (EB): 50 mM Hepes pH 8.0, 300 mM NaCl, 5 % glycerol, 250 mM imidazole pH 8.0.

Procedure: Total volume of Ni-NTA added to BioRad drip column: 4 mL (50 %). Resin washed with 12.5 mL of WBI. The supernatent was applied to a column using 5 mL pipette and allowed to pass over the resin. The flow through was collected in a 50 mL falcon tube and applied once more to the column. Two wash steps followed. Wash with 12.5 mL of WBI. Wash with 12.5 mL column vols of WBII. Elute with 14 mL of EB into 7x2 mL fractions.

Column 2 : Size exclusion using a S75 16/60 column

Buffers : GF Buffer: 50 mM Tris pH 8.0, 500 mM NaCl, 0.5mM TCEP

Procedure: The column was pre-equilibrated with two column volumes of GF buffer (flow rate 1 mL/min). The fractions from gel filtration that contained RGS 1A were pooled and concentrated before loading on to S75 16/60 gel filtration column. The fractions containing protein were identified on a coomasie blue stained gel.

Extraction

Procedure
1 tablet protein inhibitor in 10 mL Lysis Buffer was added to the 1L growth pellet. Total vol: 45 mL (estimate).

Cell breakage: 5 passes through the Emulsiflex C5 high pressure homogeniser. Total vol: 50 mL (estimate).
Concentration:
MassSpec:
Crystallization: Column 1: Low pressure chromatography using Bio-Rad Econo column (2.5 cm x 13 cm).

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NMR Spectroscopy:
Data Collection:
Data Processing: