Entry Clone Source: Origine

Entry clone accession: gi|18860900

SGC Construct ID: PTPRJA-c012

GenBank GI number: gi|18860900

Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]

Tags and additions: Tag sequence: mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.

Final protein sequence:

The expressed sequence of **2CFV** corresponds to the wild type sequence in which the WPD motif has been replaced by APD (highlighted in sequence in red). This mutant is still active using fluorescent based substrates:

mhhhhhhssgvdlgtenlyfq*smKLIRV
ENFEAYFKKQQADSNCGFAEEYEDLKLVG
ISQPKYAAELAENRGKNRYNNVLPYDISR
VKLSVQTHSTDDYINANYMPGYHSKKDFI
ATQGPLPNTLKDFWRMVWEKNVYAIIMLT
KCVEQGRTKCEEYWPSKQAQDYGDITVAM
TSEIVLPEWTIRDFTVKNIQTSESHPLRQ
FHFTS**A**PDHGVPDTTDLLINFRYLVRDYM
KQSPPESPILVHCSAGVGRTGTFIAIDRL
IYQIENENTVDVYGIVYDLRMHRPLMVQT
EDQYVFLNQCVLDIVRSQKDSKVDLIY

In addition we generated a mutant (**2NZ6**) in which the active site cysteine (marked in red) was substituted by a serine (C/S mutant):

mhhhhhhssgvdlgtenlyfq*smKLIRV
ENFEAYFKKQQADSNCGFAEEYEDLKLVG
ISQPKYAAELAENRGKNRYNNVLPYDISR
VKLSVQTHSTDDYINANYMPGYHSKKDFI
ATQGPLPNTLKDFWRMVWEKNVYAIIMLT
KCVEQGRTKCEEYWPSKQAQDYGDITVAM
TSEIVLPEWTIRDFTVKNIQTSESHPLRQ
FHFTSWPDHGVPDTTDLLINFRYLVRDYM
KQSPPESPILVH**S**SAGVGRTGTFIAIDRL
IYQIENENTVDVYGIVYDLRMHRPLMVQT
EDQYVFLNQCVLDIVRSQKDSKVDLIY

Host: BL21 (DE3)

Growth medium, induction protocol: 1ml from a 10 ml LB overnight culture containing 50 µg/ml kanamycin was used to inoculate 1 liter of LB media containing 50 µg/ml kanamycin. Cultures were grown at 37°C until the OD 600 reached ~0.3. After that the temperature was adjusted to 18°C. Expression was induced for 4 hours using 1mM IPTG at an OD 600 of 0.8. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen. Binding buffer: 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol.

Extraction buffer, extraction method: Cell pellets were lysed using a high pressure cell disrupter. The lysate was centrifuged at 50,000 rpm for 40 minutes and the supernatant collected for purification.

Column 1: Ni-affinity chromatography.

Buffers: Binding buffer: 50 mM HEPES pH 7.5, 500mM NaCl, 5% Glycerol. **Wash buffer:** 50 mM HEPES pH 7.5, 500mM NaCl, 20mM Imidazole, 5% glycerol. **Elution buffer:** 50mM HEPES pH 7.5, 500mM NaCl, 50 to 250mM Imidazole, 5% Glycerol.

Procedure: 5 ml of 50% Ni-NTA slurry (Qiagen) was applied to a 1.5 x 10 cm gravity column. The column was equilibrated with 30 ml binding buffer. The lysate was applied to the column which was subsequently washed with 3 x 10 ml wash buffer. PTPRJ was eluted by a step gradient generated by 5 ml portions of elution buffer with increasing concentration of imidazole (concentrations used: 50mM, 100mM, 250mM). The eluted protein fractions were collected and analyzed by SDS - PAGE . After elution DTT was added to a final concentration of 10mM.

Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)

SEC -Buffers: 10 mM Hepes, pH 7.5, 25 mM NaCl.

Procedure: The fractions eluted of the Ni-affinity chromatography were pooled and concentrated to about 1 ml using Centricon concentrators (10kDa cut off). The concentrated protein was applied to a Superdex S75 column equilibrated in SEC buffer at a flow rate of 1 ml/min. Eluted fractions were 95% pure as judged by SDS - PAGE .

Protein concentration: Centricon with a 10kDa cut off in SEC -buffer

Crystallization:
W/A mutant (2CFV): Crystals were obtained using the vapor diffusion method and a protein concentration of 9 mg/ml by mixing 100nl of the concentrated protein with 100nl of a well solution containing 0.01M $NiCl_2$; 0.1M TRIS pH 8.5 and 1M Li_2SO_4. 1 mM of the peptide IGEHpYVHVNA corresponding to the Met-receptor were added to the protein prior to crystallization.

C/S mutant (2NZ6): Crystals were obtained using similar condition as described for the W/A mutant with a protein concentration of 9 mg/ml

Data Collection: Resolution: 2.5Å (2CFV) and 2.3Å (2NZ6); Crystals were cryo-protected using the well solution and 25% ethylene glycole and flash frozen in liquid nitrogen. **X-ray source:** Diffraction data were collected at the SLS beamline X10 at a single wavelength (0.979 Å).