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| Target information | Target ID: JMJD3A  SGC construct ID: JMJD3A-c031 Purification ID: JMJD3A-p033 Entry clone source: synthetic Entry clone accession: n/a Vector: pNH-TrxT Tag and additions: Thioredoxin and 6xHis-tag followed by TEV cleavage site(\*) MHHHHHHSSGMSDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLAGTENLYFQ\*  Protein sequence: \*SMDVVRASRNAKVKGKFRESYLSPAQSVKPKINTEEKLPREKLNPPTPSIYLESKRDAFSPVLLQFCTDPRNPITVIRGLAGSLRLNLGLFSTKTLVEASGEHTVEVRTQVQQPSDENWDLTGTRQIWPCESSRSHTTIAKYAQYQASSFQESLQEEKESEDEESEEPDSTTGTPPSSAPDPKNHHIIKFGTNIDLSDAKRWKPQLQELLKLPAFMRVTSTGNMLSHVGHTILGMNTVQLYMKVPGSRTPGHQENNNFCSVNINIGPGDCEWFAVHEHYWETISAFCDRHGVDYLTGSWWPILDDLYASNIPVYRFVQRPGDLVWINAGTVHWVQATGWCNNIAWNVGPLTAYQYQLALERYEWNEVKNVKSIVPMIHVSWNVARTVKISDPDLFKMIKFCLLQSMKHCQVQRESLVRAGKKIAYQGRVKDEPAYYCNECDVEVFNILFVTSENGSRNTYLVHCEGCARRRSAGLQGVVVLEQYRTEELAQAYDAFTLVRARR Host: BL21(DE3)-SlyD |
| Expression method | **Media:**  Starter culture: 2 x LB + 50 µg/mL Kanamycin + 34 µg/mL chloramphenicol  Expression culture: 2 x 1L home-made TB + 50 µg/mL Kanamycin + salts added after autoclaving.  **Induction protocol:**  Glycerol stock BL21(DE3)-R3-pRARE2  bacteria carrying plasmid was used to inoculate 20ml of starter culture. The next day each litre of TB in 3L baffled flasks was inoculated with 10 mL of starter culture and grown at 37°C. After OD reached 0.6 and incubator was cooled to 18oC expression was induced with 0.3 mM.    **Osmotic shock protocol:**  Bacteria were harvested using JLA8.1 rotor at 5krpm for 15min at 4C. Pellet was resuspended in 70ml of 50mM HEPES, 1mM EDTA, 20% sucrose and spun using JLA 16.25 at 9000xg for 20min. Pellet resuspended again in 5mM Mg2SO4 incubated with shaking for 10min on ice and spun for another 20min at 4500xg. |
| Extraction | **Extraction buffers:**  Lysis buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 20 mM imidazole, protease inhibitor cocktail set III (Calbiochem), 25U of benzonase (EMD Milipore)  **Extraction procedure:**  After osmotic shock pellet was in lysis buffer. The cells were disrupted by high pressure homogenisation (25 kpsi). Cell debris were removed by centrifugation for 45 minutes at 35 000xg |
| Purification | **Column 1**  IMAC:   HisTrap FF Crude, 1 mL (GE/Amersham Biosciences) equilibrated in lysis buffer  Elution buffer 50 mM HEPES pH 7.5, 500 mM NaC, 250 mM imidazole,  5% glycerol.  **Procedure:**  The cell extract was loaded on the column at 1 mL/minute on an AKTA-express system (GE/Amersham). The column was then washed with 30 column volumes of lysis buffer and proteins eluted with elution buffer.  Elution fractions were pooled and 1mM ETDA together with TEV were added, incubated overnight at 4oC.  **Column 2**  Ion exchange: 1ml HiTrap HP SP ion exchange column (GE/Amersham Biosciences) equilibrated in low salt buffer Low salt buffer: 25 mM HEPES pH 7.5, 50 mM NaCl High salt budder: 25 mM HEPES pH 7.5, 1M NaCl Dilution buffer: 25mM HEPES pH 7.5  **Procedure:**  Cleaved protein was diluted with dilution buffer 10x (50mM NaCl) and loaded on ion exchange columns at flow of 1ml/min.  Resin was washed with 60CV of low salt buffer and protein eluted with 0-15% 20CV (additional wash), 35%B 50CV gradient at flow 0.8ml/min. Protein start to elute at 20%B.  Glycerol and NaCl were added to final concentration of 5% anf 0.5M respectively. |
| Concentration and storage | The protein was concentrated using an Amicon Ultracel centrifugal concentrator (50 kDa MWCO) to 1 mg/ml by A280 and predicted extinction coefficient and kept in -80oC. |
| Mass spec and activity assay | Mass has been confirmed by mass spectrometry and oxidation/sodium adducts has been detected. Activity confirmed by FDH assay. |