

# Cp 14-3-3 ε

**PDB:**2NPM

## Revision

**Revision Type:**created

**Revised by:**created

**Revision Date:**created

**Entry Clone Accession:**cgd3\_1290 (CryptoDB.org)

**Entry Clone Source:**Cp gDNA Iowa strain

**SGC Clone Accession:**

**Tag:**N-Terminal hexahistidine tag with integrated tobacco etch virus (TEV) protease cleavage site.

**Host:**E. coli BL21 (DE3) Rosetta-R3

## Construct

**Prelude:**

**Sequence:**

mgsshhhhhhssgreenlyf fqgILRTQHTIRDSENIKNSKMSDSVNARESNVYMAKLAEQAQERYDEMAKYMKDVEARQESEELTVE  
ERNLLSVAYKNAVGSRSSWRIISSVEQKEHSRNAEDASKMCGKYRSKVEAE LTDICNDILTMKHLIPTATSPDSKVFYFKMKGD  
YHRYISEFSTGDSKQSSAEDALKAYKDATVVAKDLLEPTHPIRLGLALNFSVFHYEILNEPRAAIDMAKEAFEMATEQLDKLSEDCYK  
DSTLIMQLLRDNLTWTAD

**Vector:**p15-TEV-LIC

## Growth

**Medium:**

**Antibiotics:**

**Procedure:**Cryptosporidium parvum putative 14-3-3 protein was expressed in E. coli BL21 (DE3) Oxford in Terrific Broth (TB) in the presence of ampicillin and chloramphenicol (100 µg/mL and 34 µg/mL respectively). A single colony was inoculated into 50 mL of LB with ampicillin and chloramphenicol in a 125 mL flask and incubated with shaking at 250 rpm overnight at 37 °C. The culture was transferred into 2 X 1.8 L TB with ampicillin and chloramphenicol and 0.3 mL of antifoam (Sigma) in 2 L bottles and cultured using the LEX system to an OD600 of 4.0. The culture was cooled to 15 °C, and isopropyl-1-thio-D-galactopyranoside (IPTG) was added to 0.4 mM, and the culture was incubated overnight at 15 °C.

## Purification

**Procedure**

Column 1: The cleared cell lysate was loaded onto a column containing 10 g DE-52 resin

(Whatman), and then directly onto a 2.5 mL Ni-NTA (Qiagen) column at approximately 1.5 mL/min. When all the lysate was loaded, the two column system was washed with 15 mL binding buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM imidazole, and 5 % glycerol) at 2 mL/min. After washing, the protein was eluted from the Ni-NTA column with 15 mL of Elution Buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, and 5 % glycerol). EDTA was added immediately to 1 mM; and DTT was added to 1 mM 15 minutes later.

**TEV Protease Cleavage:** The eluted Cp 14-3-3 protein was treated with TEV protease in a 1:500 molar ratio of protease:protein, and incubated at 4°C overnight concurrent with dialysis against 10 mM HEPES, pH 7.5, 500 mM NaCl, and 5 mM imidazole. The cut protein was separated from the uncut protein by passage through another 2.5 mL Ni-NTA column.

**Column 2:** The cut Cp 14-3-3 was applied to a Sephadex S200 26/60 gel filtration column (GE Healthcare) pre-equilibrated with 10 mM HEPES, pH 7.5 and 500 mM NaCl. The collected fractions corresponding to the eluted protein peak were concentrated to 10 mg/mL using a 15 mL Amicon Ultra centrifugal filter device (Millipore). Aliquots of the purified Cp 14-3-3 protein were stored at -70 °C.

## Extraction

### Procedure

The culture was harvested by centrifugation and the cell pellet was suspended in 160 mL of binding buffer (50 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, and 5 mM imidazole) with protease inhibitor (1 mM benzamidine-HCl and 1 mM phenylmethyl sulfonyl fluoride, PMSF) and kept in 50 mL Falcon tubes at  $\Delta$  80 °C. Before purification, the cell suspension was thawed overnight at 4 °C. Prior to mechanical lysis, each tube of cell suspension was pretreated with 0.5 % CHAPS and 500 units of benzonase (per 40 mL of resuspended cell pellet) for 40 minutes at room temperature. Then the cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18000 psi. The lysate was centrifuged at 24000 rpm for 20 minutes at 10 °C.

**Concentration:** 10 mg/mL

### Ligand

### MassSpec:

**Crystallization:** Purified Cp 14-3-3 was first treated with 2 mM DTT and a three fold molar excess of consensus peptide 2  $\Delta$  RAI(pS)LP where pS denotes a phosphoserine  $\Delta$  followed by incubation on ice for 1 h. The protein:peptide complex was crystallized using the hanging drop vapour diffusion method in a 24-well Linbro plate. The protein solution (1  $\mu$ L) was added to 1  $\mu$ L reservoir buffer containing 14% PEG3350, 0.1 M Calcium Acetate, 0.2 M trimethylamine-N-oxide, and 0.1 M HEPES pH 7.0, placed over 500  $\mu$ L reservoir buffer, and incubated for at 293 K. Diamond shaped crystals (100-200  $\mu$ m) grew to maximum size in two days.

### NMR Spectroscopy:

### Data Collection:

### Data Processing: