# DTX3L

**PDB:**3PG6
**Entry Clone Accession:**AU91-A12
**Entry Clone Source:**Mammalian Gene Collection
**SGC Clone Accession:**dtxl3.0601-0740.204F05 (SDC204F05)
**Tag:**N-terminal: MGSSHHHHHHSSGLVPRGS
**Host:**Competent BL21 (DE3) cells (Invitrogen, C6000-03)
**Vector:**pET28aLIC vector (GenBank, EF442785)

**Sequence:**

MGSSHHHHHHSSGLVPRGSTSYGIQKGNQPEGSMVFTVSRDSLPGYESFGTIVITYSMKA
GIQTEEHPNPGKRYPGIQRTAYLPDNKEGRKVLKLLYRAFDQKLIFTVGYSRVLGVSDVIT
WNDIHHKTSRFGGPEMYGYPDPSYLKRVKEELKAKGIE

# Growth

**Medium:** TB (Sigma, T0918) supplemented with 150 mM glycerol, 100 µM antibiotic and 600 µl antifoam 204 (Sigma A-8311)
**Procedure:**Competent BL21 (DE3) cells (Invitrogen, C6000-03) were transformed and grown using the LEX system (HarbingerBiotech) at 37 °C in 1L bottles (VWR, 89000-242) containing 900 ml of growth medium. When the $OD_{600}$ reached a value of about 6.0, the temperature was reduced to 15 °C, and one hour later the culture was induced with 1 mM IPTG (BioShop, IPT001) and incubated overnight (16 hours) at 15 °C. To produce the SeMet derivative, the same bacterial cells were grown using M9 SeMet High-Yield growth media kit package (Medicilon, MD045003) according to manufacturer's instructions.

# Purification

**Procedure:**
Protein was purified using the Streamline purification system [1]. Briefly, bacterial cultures were directly microfluidized with the outflow connected to a novel column assembly, containing IMAC beads; a few hundred milliliters of regular buffer was also fluidized to wash the beads, and columns were eluted with imidazole by gravity. To each 1L culture bottle the following was sequentially added: NaOH (final pH ~7.5), imidazole (final 8mM), and BME (final 1 mM). Each bottle was fluidized through a Microfluidizer (model M110-EH with H10Z ceramic chamber performed at about 15,000 psi) with the outflow directly connected to a special nozzled-column containing 3 ml of settled HisLink (Promega, V8821). Approximately 200 ml fluidizer buffer was also fluidized to wash the beads. Columns were gravity-washed with 20 mL wash buffer, and protein was gravity-eluted with 8.0 ml elution buffer. Eluate was dialyzed overnight at 4 oC against about 100 volumes of dialyses buffer. Samples were concentrated using a 10 KDa MW cut-off concentrator (Millipore, UFC900524) at 3500 xg to a final value of ~12 mg/ml. Protein yield was 5 mg per liter of bacterial culture.

Alenkin D., Yermekbayeva L., Mujib S., Vesterberg A., Newman E., Yamazaki K., Cossar D., and Dhe-Paganon S. - "A centrifugation-free high-throughput protein purification system using in-line microfluidization" *Protein Expr Purif.* May 14 2011.

# Structure Determination

**MassSpec:** MW = 17864.22 g/mol
**Crystallization:** Crystals were grown at 20 oC in hanging drop plates (Hampton, HR3-170) by mixing equal volumes of protein (12 mg/mL) and Crystallization Buffer [(22% PEG 3350, 200mM Tri-Lithium Citrate, pH 6.0 for native data @1.7Å), (22% PEG 3350, 200mM Tri-Lithium Citrate, pH 5.0 for seleno-methionine data @2.0Å)]. Suitable crystals were cryoprotected by immersion in well solution supplemented with 8 % (v/v) glycerol prior to dunking and storage in liquid nitrogen.